

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
26 April 2001 (26.04.2001)

PCT

(10) International Publication Number
WO 01/29262 A2

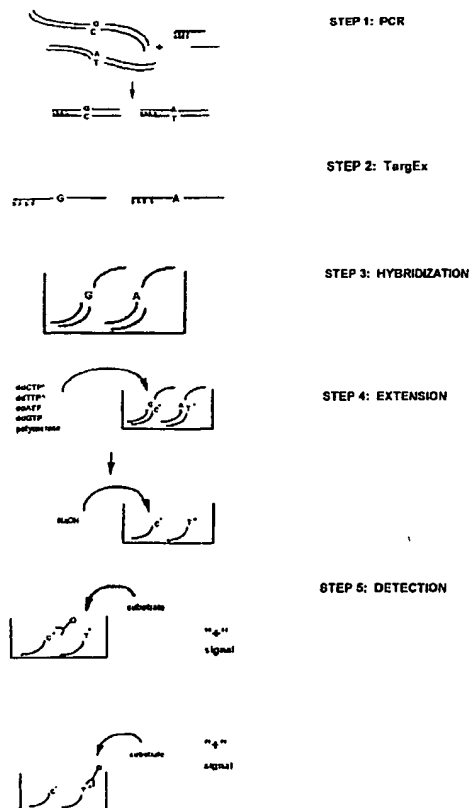
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- (22) International Filing Date: 13 October 2000 (13.10.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 60/160,096 15 October 1999 (15.10.1999) US (74) Agents: RAMOS, Robert, T. et al.; Campbell & Flores LLP, 7th floor, 4370 La Jolla Village Drive, San Diego, CA 92122 (US).
- (63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application: US 60/160,096 (CIP) Filed on 15 October 1999 (15.10.1999) (81) Designated States (national): AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EE, ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL,

[Continued on next page]

(54) Title: GENOTYPING REAGENTS, KITS AND METHODS OF USE THEREOF

(57) Abstract: The present invention provides oligonucleotides that can be used to determine the presence, absence or identity of a single nucleotide polymorphism (SNP), kits containing such oligonucleotides, and methods of genotyping a nucleic acid sample using such oligonucleotides.

Diagram of GBA Process



WO 01/29262 A2



IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

GENOTYPING REAGENTS, KITS AND METHODS OF USE THEREOF

Background

Sequencing the human genome has provided information to dramatically change the way diseases are studied, diagnosed and treated. The fields of pharmacogenomics and pharmacogenetics focus on the variability and diversity of DNA, and how this diversity can impact biology, including response to drugs. The most common form of genetic diversity is thought to come in the form of individual DNA bases being different than the corresponding base in the average population. These single nucleotide polymorphisms ("SNPs") are a natural form of mutation, which are hereditary in nature. SNPs are viewed as both direct and indirect markers for many human diseases. Therefore, the analysis, or "scoring," of SNPs to determine their role in disease and drug efficacy can lead to the development of powerful diagnostics and therapeutics.

Adverse drug reactions (ADRs) represent the leading cause of hospitalization and mortality in the U.S., resulting in over 1.5 million hospitalizations and 100,000 deaths per year (J. Lazarou, et.al. in *The Journal of the American Medical Association (JAMA)*, (1998, 1200-1205). In addition, many promising drugs developed by pharmaceutical companies never make it through clinical development because of genetically based adverse reactions (ADRs) in a small number of patients. Therefore, scoring SNPs can identify at risk patients before the drug is prescribed and salvage potentially

useful new drugs. Alternatively, patients with SNP-induced variations in their conditions, evident in diseases like hypertension, can be identified and then prescribed the drug most appropriate for their genetic variant. Therefore, SNP analysis will be used to streamline drug discovery and development leading to safer and more effective tailor-made drugs.

Accordingly, new reagents are needed that can be used to identify whether heretofore unknown SNPs are present or absent from a given nucleic acid sample. The present invention satisfies this need and provides related advantages as well.

Summary of the Invention

In accordance with the present invention, there are provided oligonucleotides that can be used to determine the presence, absence or identity of a single nucleotide polymorphism. Invention oligonucleotides include genotyping oligonucleotides that hybridize with a portion a nucleic acid sequence listed in column 4 of Table 1, or complementary sequence thereof, and amplification oligonucleotides.

In accordance with another embodiment of the invention, there are provided kits useful for determining the presence, absence or identity of a single nucleotide polymorphism. In one embodiment, an invention kit comprises an oligonucleotide that hybridizes with a portion of a nucleic acid sequence listed in column 4 of Table 1, or complementary sequence thereof. In another embodiment, an invention kit comprises amplification oligonucleotides of columns 1 and 2 of Table 1. Also provided is an invention kit comprising an

oligonucleotide that hybridizes with a portion of a nucleic acid sequence listed in column 4 of Table 1, or complementary sequence thereof and two amplification primers.

5 Another embodiment of the invention provides methods of genotyping a nucleic acid sample by hybridizing a genotyping oligonucleotide to a nucleic acid sequence listed in column 4 of Table 1, or complementary sequences thereof. The genotyping
10 oligonucleotide can then be employed in a variety of reactions, such as, for example, a primer extension reaction. Also provided is a method of genotyping a nucleic acid sample by amplifying a target nucleic acid sequence and performing a single-nucleotide primer
15 extension reaction employing a genotyping oligonucleotide that hybridizes to the target nucleic acid sequence.

Brief Description Of The Figures

Figure 1 shows a schematic for genotyping a heterozygous nucleic acid sample containing two target
20 nucleic acids using the particular primer extension reaction referred to as Single Nucleotide Primer Extension (SNPE) and/or GBA. In step 1, genomic DNA is amplified using a pair of PCR primers from columns 1 and 2 of the same row of Table 1 herein, in which the five
25 most 5' nucleotides of one of the two PCR primers are linked by four exonuclease-resistant phosphorothioated linkages (e.g., 5' C-(p)-G-(p)-C-(p)-A-(p)-GTCTCAGGCCAGCT 3', for SEQ ID NO:2, where "-(p)-" represents a phosphorothioate linkage). The PCR primers are
30 complementary to sequences flanking either side of a specific nucleotide site (e.g., the site of a SNP) in the genomic DNA, thus resulting in an amplified nucleic acid

(the target nucleic acid) containing this specific site. In step 2, the amplified PCR product target nucleic acid is subjected to T7 gene 6 exonuclease digestion, which removes the strand of the target nucleic acid that did not include the 5' phosphorothioated linkages, resulting in a single-stranded target nucleic acid. In step 3, the genotyping primer (or SNPE primer) is hybridized with the single stranded target nucleic acid such that the specific nucleotide of the target nucleic acid that is to be analyzed (i.e., the SNP) is not hybridized, and is immediately adjacent the 3' end of the genotyping primer. In step 4, a terminator reagent mixture comprising dideoxy terminators corresponding to A, C, G and T, together with a polymerase, are added to the target nucleic acid-genotyping primer complex. The polymerase extends the genotyping primer by a single base, consisting of one of the four terminators, where the added terminator base is complementary to the SNP to be determined. The terminator base that is added to the genotyping primer is labeled with a detectable marker. In the second part of step four, NaOH is added to dissociate the target nucleic acid strand which is washed away, while the genotyping primer, fixed to a solid support, remains. In step 5, a detection reagent is added, if necessary, to generate signal from the detectable marker. This can be performed in an ELISA format where an enzyme-linked antibody specific for the detectable marker of a terminator is contacted with the extended genotyping primer. When detectable markers that can be distinguished from each other are used, multiple alleles can be identified in a single well. For example, as illustrated in step 5, two alleles can be identified (C and T) by the sequential use of antibodies selective for each of the terminators (ddCTP and ddTTP).

Figures 2A and 2B show schematics for genotyping a homozygous nucleic acid sample using the Single Nucleotide Primer Extension (SNPE) by the enzyme-linked immunosorbant assay (ELISA) detection method after amplification using a phosphorothioated primer and exonuclease digestion. In figure 2A, the template strand indicated in the figure is a representative target nucleic acid molecule (such as those listed in SEQ ID NO:4n+4). The SNPE primer indicated in the figure is a representative genotyping primer (such as those listed in SEQ ID NO:4n+3). The nucleotide to be interrogated is indicated as the SNP to be typed. Primer extension is performed as described herein, in which the polymerase extension step can be performed in the presence of the terminators ddATP, ddGTP, fluorescein-ddCTP and biotin-ddUTP. The last two terminators are linked to detectable markers. Figure 2B shows the detection step after the single nucleotide primer extension results in the addition of fluorescein-ddCTP onto the 3'-end of the SNPE primer. The diagram illustrates the interaction of alkaline phosphatase-conjugated anti-fluorescein antibody and the generation of signal at OD₄₀₅ after the addition of PNPP (p-nitrophenyl phosphate) substrate. Since the biotin-ddUTP is not incorporated, the anti-biotin horse radish peroxidase (HRP) does not bind to the primer, no reaction with the TMB (tetramethylbenzidine) substrate occurs and, hence, no signal is generated at OD₆₂₀.

Figure 3 shows a scatterplot of OD₄₀₅ (X-axis) versus OD₆₂₀ (Y-axis) resulting from SNPE that demonstrates a result that is heterozygous. The PCR primers used were oligonucleotides of SEQ ID NOs:25 and 26. The SNPE primer used was an oligonucleotide with SEQ ID NO:27. The targeted nucleic acid with the suspected polymorphism contains the sequence of SEQ ID NO:28. SNPE

was performed using fluorescein-ddCTP and biotin-ddUTP. The Y-axis corresponds to signal obtained from ddUTP incorporation and the X-axis corresponds to signal obtained from ddCTP incorporation. Each circle (●) indicates a result with a specific test sample (unknown) from an individual genomic DNA. Plus (+) symbols are positive controls using a synthetic template. Crosses (x) are negative controls where no PCR is performed.

10 Detailed Description of the Invention

In accordance with the present invention, there are provided oligonucleotides that can be used to determine (genotype) the presence, absence or identity of a single nucleotide polymorphism (SNP) at a preselected site in a human genomic nucleic acid sample. The location of the SNP in each SNP-containing oligonucleotide set forth in column 4 of Table 1 (also referred to herein as SEQ ID NO:4n+4, wherein n=0 through 934) herein corresponds to the nucleotide position on each SEQ ID NO:4n+4 that is immediately adjacent the nucleotide complementary to the 3' end of the corresponding genotyping oligonucleotide set forth in column 3 of the same row of Table 1. The nucleotide set forth on SEQ ID NO:4n+4 corresponding to the SNP site corresponds to the predominant polymorphism.

As set forth in Table 1, each row of oligonucleotides disclosed corresponds to a set of oligos useful for genotyping a nucleic acid sample for the presence or absence, or identity of a particular SNP. The oligonucleotides set forth in columns 1 and 2 of Table 1 (also referred to herein as SEQ ID NO:4n+1 and SEQ ID NO:4n+2, respectively, where n=0 through 934) are

used as primers to amplify the nucleic acid sample having the corresponding SNP-containing oligonucleotide from the same row in Table 1 (referred to as SEQ ID NO:4n+4, where n=0 through 934). See Figure 1. Once the nucleic acid sample having the corresponding SNP-containing oligonucleotide has been amplified, the corresponding genotyping oligonucleotide from column 3 (referred to as SEQ ID NO:4n+3, where n=0 through 934) of the same row in Table 1 is used to genotype the target nucleic acid, preferably by a primer extension reaction.

As used herein, the term "oligonucleotide", "oligo" or grammatical variations thereof, refers to polynucleic acid that can be either DNA or RNA. Invention oligos can be produced by methods well-known in art such as described in the Examples hereinafter; or the oligos can be purchased from commercial oligonucleotide production companies.

As used herein, the phrase "SEQ ID NO:4n+3, wherein n=0 through 934" refers to the group of oligonucleotides in the Sequence Listing that are set forth in column 3 of Table 1 herein (where n=(row number - 1). For example, n=0 corresponds to SEQ ID NO:3, n=1 corresponds to SEQ ID NO:7, ..., n=934 corresponds to SEQ ID NO:3739 of the Sequence Listing. Likewise, SEQ ID NO:4n+1, SEQ ID NO:4n+2, and SEQ ID NO:4n+4, wherein n=0 through 934, refers to the groups of oligonucleotides set forth in columns 1, 2 and 4, respectively, of Table 1 herein.

The oligonucleotides set forth in column 3 of Table 1 (SEQ ID NO:4n+3, where n=0 through 934) are also referred to herein as genotyping oligonucleotides. In addition, for each SNP located in each oligonucleotide

set forth in column 4 of Table, there are at least four potential genotyping oligonucleotides (e.g., genotyping primers) that are hybridizable to the region one nucleotide removed from either 5' or 3' end of the SNP nucleotide (two for each strand). Two of the genotyping primers (one for each target nucleic acid strand) are complementary to the target nucleic acid and have their 3' ends one nucleotide 3' to the SNP nucleotide. These primers can be used, for example, in a polymerase primer extension reaction such as described in Goelet, P. et al., U.S. Pat No.5,888,819 and Nikiforov, T.T. et al., U.S. Pat. No. 5,518,900, which are herein incorporated by reference. The other two primers are complementary to the target nucleic acid and have their 5' ends one nucleotide 5' to the SNP nucleotide. These primers can be used in, for example, a ligase/polymerase genotyping reaction such as described in Nikiforov, T.T. et al., U.S. Pat. No. 5,679,524, or a nucleotide and terminator mixture reaction such as described in Soderlund et al., U.S. Pat. No. 6,013,431 or by Koster et al., U.S. Pat. No. 6,043,031, which is herein incorporated by reference.

In addition, the oligonucleotides set forth in column 3 of Table 1 (SEQ ID NO:4n+3, where n=0 through 934) can be made one nucleotide longer such that the nucleotide at the 3' end lies complementary to the SNP nucleotide. These primers can be used in alternate genotyping methods, for example, in allele-specific polymerase chain reaction (AS-PCR) (Newton et al., 1989, Nucl Acids Res 17:2503-2516), ligase chain reaction (LCR) (Barrany, 1991, PNAS, USA, 88: 189-193), or oligonucleotide ligation assay (OLA) (Landegren et al., 1988, Science 241:1077), where the 3' nucleotide of the genotyping primer determines the presence of the SNP nucleotide.

In addition, the oligonucleotides set forth in column 3 of Table 1 (SEQ ID NO:4n+3, where n=0 through 934) can be made one or more nucleotides shorter such that the genotyping primers are complementary to the target nucleic acid and have their 3' ends two or more nucleotides 3' to the SNP nucleotide. These primers can be used in, for example, a labeled nucleotide and terminator mixture reaction such as described in Soderlund et al., U.S. Pat. No. 6,013,431 or by Koster et al., U.S. Pat. No. 6,043,031, which is herein incorporated by reference.

The genotyping primer can be an oligodeoxyribonucleotide (DNA), an oligoribonucleotide (RNA), or a copolymer of deoxyribonucleotides and ribonucleotides. The genotyping primer can be either natural or synthetic. The genotyping primer can be synthesized either enzymatically in vivo, enzymatically in vitro, or non-enzymatically in vitro. The genotyping primer can include modifications of the ribose-phosphate backbone, such as phosphorothioate or methylphosphonate or other backbone modifications such as peptide nucleic acid (PNA). The genotyping primer can also include modifications of the nucleotide bases. The genotyping primer can be labeled with a detectable marker; this detectable marker can be different from any detectable marker present in the reaction mixture or any detectable marker attached to the target nucleic acid. In addition, for use with single-nucleotide primer extension reactions, the genotyping primer must be capable of hybridizing or annealing with nucleotides present in the target nucleic acid, immediately adjacent to, and upstream of, the nucleotide base to be identified. One way to accomplish the desired hybridization is to have the template-dependent primer be substantially

complementary or fully complementary to the known base sequence immediately adjacent to the base to be identified. The genotyping primer may be bound to a solid surface such as described in U.S. Pat. No. 5 5,610,287, incorporated herein by reference in its entirety. Multiple different genotyping primers can be immobilized on a surface to create an array, thus allowing the determination of multiple genotypes in a single reaction. Multiple different genotyping primers 10 with distinct attachment means ("tags") can be used for multiplex single nucleotide primer extension in solution and subsequently separated for discrete detection, such as on a nucleic acid array comprising nucleic acids complementary to the attachment means of the genotyping 15 primers ("tag arrays").

Genotyping oligonucleotides of the invention can be as long as the nucleotide distance between an amplification primer and the SNP nucleotide, preferably not overlapping the amplification primer sequence, 20 preferably less than 90 nucleotides, preferably less than 50 nucleotides, more preferably between 14 and 50 nucleotides, most preferably between 17 and 30 nucleotides.

When invention genotyping oligonucleotides are 25 used in, for example, single-primer-extension reactions, hairpin loop structures can form due to self-complementarity of the nucleotide sequences of the oligo/primer. In order to disrupt the hairpin loop structure formation, "single-nucleotide-spacers" can be 30 inserted in the midst of the hairpin loop structure. As used herein, the term "single-nucleotide-spacer" refers to any non-Watson-Crick moiety incorporated within an oligonucleotide for the purpose of weakening nucleic acid

duplex stability without altering the fidelity of the surrounding Watson-Crick base pairs. Thus, modifications to genotyping oligonucleotides contemplated herein are for the purpose of destabilizing duplex formation, specifically disfavoring intrastrand folding in favor of interstrand pairing. The location of single-nucleotide-spacers are set forth in the Sequence Listing and column 3 of Table 1 as an "X". Such moieties are well-known and available in the art and can include anucleosidic moieties, abasic moieties, non-naturally occurring nucleotide analogs, and non-Watson/Crick base moieties.

As used herein, the term "anucleosidic moieties" refers to moieties that approximate the spacing of the phosphoribose linkage. Thus, anucleosidic sites are contemplated herein as space-holders between neighboring phosphates along the oligo backbone. Examples of anucleosidic moieties for use in invention oligonucleotides include phosphoramidite-coupled multicarbon aliphatic chains that lack a nucleotide base and approximate the internucleotide spacing. For example, a C-3 linker used in DNA synthesis (3-(4,4'-Dimethoxytrityloxy)propyl-1-phosphoramidite) (Glen Research, Sterling, VA) will have the approximate spacing of one nucleotide within a nucleic acid chain. Other anucleosidic sites include ethyl-like C2 and C1 linkers, rather than C3. C2- and C3-linkers are preferred as single-base length analogs. Larger anucleosidic spacers are contemplated herein having stretches of a few C2 or C3 linkers, or by incorporating a single C6 linker (for a two-base gap), or C9 (for 3 bases), and so forth up C18 linkers, and the like.

As used herein, the phrase "abasic moiety" refers to moieties that approximate the conformation of a

nucleotide but are chemically distinct from nucleotides, which include sugars or sugar analogs without base moieties. For example, an abasic site consists of the sugar-phosphate backbone without the base, such as
5 tetrahydrofuran-phosphate (dSpacer; Glen Research, Sterling, VA), can be used as a nucleotide analog. Other backbones include those in which one or two of the non-bridging oxygen atoms of the phosphate moiety of a nucleotide have been replaced with a sulfur-containing
10 group (especially a phosphorothioate), an alkyl group (especially a methyl or ethyl alkyl group), a nitrogen-containing group (especially an amine), and/or a selenium-containing group. In addition, peptide nucleic acids (PNAs) (Buchardt O, *Trends Biotechnol* 1993
15 Sep;11(9):384-6) or a carbohydrate are alternate backbones that can be used. It is contemplated that abasic modifications of these backbone types can be used as nucleotide analogs.

As used herein, "non-natural base variations"
20 are non-canonical bases often referred to as "degenerate bases" since they exhibit some ability to base pair to any of the 4 standard bases. Exemplary non-natural base variations include, for example, "purine" and "pyrimidine" (which would be the structural scaffolds for
25 A/G and C/T, respectively), as well as fluorine-derivatized bases, and the like. Examples of other non-natural base variations include 5-nitroindole, 3-nitropyrrole, and inosine. Numerous other "natural" base modifications that result from carcinogen exposure are
30 also contemplated herein (see, e.g., Glen Research Catalog). The single nucleotide spacer can also include nucleotide ribose or deoxyribose analogs.

As used herein, the phrase "naturally occurring, non-Watson/Crick, bases" refers to any naturally occurring canonical base other than dA, dC, dG, dT. Common bases would be deoxyinosine (dI) and
5 deoxyuridine (dU). Other naturally occurring base variants can be found in tRNA sequences, and the like.

The single nucleotide spacer can also include non-complementary Watson/Crick nucleotide bases. An example of a mismatched nucleotide base would be the
10 substitution of a thymidine base with an adenine base such that the intra helical base pair would be A:A instead of T:A and hence disrupt base pairing. For example, it is well known that some non-Watson/Crick pairs have moderate stability, such as G/T pairs. For
15 any base pair, there are 6 potential mis-pairs using the standard 4 bases.

As used herein, the term "hybridize", or grammatical variations thereof, refers to the ability of two strands of nucleic acid molecules to hydrogen bond in
20 a sequence dependent manner. For example, under appropriate conditions, complementary nucleotide sequences can hybridize to form double stranded DNA or RNA, or a double stranded hybrid of RNA and DNA. Nucleotide changes in the complementary sequence that
25 disrupt base-pairing result in a decreased stability of the hybrid. These concepts are known in the art and described in Sambrook et al., Molecular Cloning: A Laboratory Manual 2nd ed. (1989), which is incorporated herein by reference.

30 Stringency levels used to hybridize a given probe with target-DNA can be readily varied by those of skill in the art. The phrase "stringent hybridization"

is used herein to refer to conditions under which nucleic acid hybrids are stable. As known to those of skill in the art, the stability of hybrids is reflected in the melting temperature (T_m) of the hybrids. In general, the stability of a hybrid is a function of GC content, cation concentration, formamide concentration and temperature. Typically, the hybridization reaction is performed under conditions of lower stringency, followed by washes of varying, but higher, stringency. Reference to hybridization stringency relates to such washing conditions.

As used herein, the phrase "moderately stringent hybridization" refers to conditions that permit target-DNA to bind a complementary nucleic acid that has about 60% identity, preferably about 75% identity, more preferably about 85% identity to the target DNA; with greater than about 90% identity to target-DNA being especially preferred. Preferably, moderately stringent conditions are conditions equivalent to hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.2X SSPE, 0.2% SDS, at 65°C.

The phrase "high stringency hybridization" refers to conditions that permit hybridization of only those nucleic acid sequences that form stable hybrids in 0.018M NaCl at 65°C (i.e., if a hybrid is not stable in 0.018M NaCl at 65°C, it will not be stable under high stringency conditions, as contemplated herein). High stringency conditions can be provided, for example, by hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.1X SSPE, and 0.1% SDS at 65°C.

The phrase "low stringency hybridization" refers to conditions equivalent to hybridization in 10% formamide, 5X Denhart's solution, 6X SSPE, 0.2% SDS at 42°C, followed by washing in 1X SSPE, 0.2% SDS, at 50°C. Denhart's solution and SSPE (see, e.g., Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989) are well known to those of skill in the art as are other suitable hybridization buffers.

As used herein, the phrase "immediately adjacent the 3' end" refers to a specific first nucleotide position, typically on the target nucleic acid or on a nucleic acid having SEQ ID NO:4n+4, or complementary sequences thereof, which is immediately 5' of a second nucleotide position on the same nucleic acid, in which the second nucleotide position is complementary to the 3'-end nucleotide of SEQ ID NO:4n+3 or complementary sequence thereof. For example, Figure 2A shows a nucleotide sequence representative of a target nucleic acid sequence or a nucleic acid sequence having SEQ ID NO:4n+4, labeled "Template strand." Figure 2A also shows a nucleotide sequence representative of a nucleic acid sequence having SEQ ID NO:4n+3, labeled "SNPE primer." The 3'-end nucleotide of the SNPE primer is an "A" which is complementary to a "T" on the Template strand. The nucleic acid immediately 5' of the "T" on the Template strand is a "G," and is labeled in the figure "SNP to be typed. Accordingly, the "G" nucleotide on the Template strand, labeled as the SNP to be typed, is "immediately adjacent the 3' end" of the SNPE primer in Figure 2A.

Preferably, the invention oligonucleotides corresponding to SEQ ID NOs:4n+1 and 4n+2, where n=0

through 934 (i.e., columns 1 and 2 of Table 1) can be used as two amplification primers for increasing the amount of target nucleic acids (set forth in column 4 of Table 1) containing the SNP. In addition, in view of the
5 target nucleic acids disclosed herein in column 4 of Table 1, those of skill in art can readily design additional amplification primers that can amplify larger target nucleic acid fragments comprising the target nucleic acids set forth in column 4. Likewise, it is
10 contemplated herein that those of skill in art can readily design additional amplification primers that can amplify smaller target nucleic acid fragments, so long as the smaller target nucleic acid comprises the SNP-site set forth in column 4. The amplification primer pairs
15 are designed such that the primers generate a target nucleic acid strand complementary to the genotyping primer and resistant to degradation, for example, by 5'-3' exonuclease.

As used herein, a nucleic acid that is
20 "resistant to degradation" or "degradation-resistant" is a nucleic acid that is resistant to chemical degradation, acid hydrolysis, base hydrolysis, or other chemical-induced hydrolysis, or is resistant to enzymatic degradation. A nucleic acid that is resistant to
25 enzymatic degradation can be resistant to exonuclease activity, endonuclease activity, and the like. In a preferred embodiment, a nucleic acid that is resistant to degradation is resistant to 5'-3' exonuclease activity, by using phosphorothioation, and the like.

30 A method of using 5'-3' exonuclease in the preparation of target nucleic acids is the subject of Nikiforov et al., U.S. Patent 5,518,900, which is herein incorporated by reference. Examples of 5'-3' exonuclease

resistant nucleic acids are disclosed in Zon, G. et al., (Anticancer Drug Design 6:539-568 (1991)) and Goodchild, J. et al. (Bioconjugate Chem. 1:613-629 (1990)), both of which are incorporated herein by reference in their
5 entirety. In general, suitable degradation-resistant nucleotide derivatives in which one or two of the non-bridging oxygen atoms of the phosphate moiety of a nucleotide have been replaced with a sulfur-containing group (especially a phosphorothioate), an alkyl group
10 (especially a methyl or ethyl alkyl group), a nitrogen-containing group (especially an amine), and/or a selenium-containing group, etc. Other modifications that confer degradation resistance such as 5'-3' exonuclease resistance can be used, for example, peptide nucleic
15 acids (PNAs) (Buchardt, O. *supra*) or 2'-O-methyl ribose modified nucleic acids Srivastava S., C. et al., U.S. Pat. No. 5,214,135. Degradation resistance is preferably achieved by the primer containing a phosphorothioate modification at the 5' end of the nucleic acid and, in a
20 preferred embodiment, containing four phosphorothioate linkages at the 5' end.

The selected nucleotide derivative is suitable for *in vitro* primer-mediated extension and provides
nuclease resistance to the region of the nucleic acid
25 molecule in which it is incorporated. In a preferred embodiment, a nucleotide derivative confers resistance to exonucleases that attack double-stranded nucleic acids from the 5'-end (5'-3' exonucleases). Examples of such exonucleases include bacteriophage T7 gene 6 exonuclease
30 and bacteriophage lambda exonuclease. Both exonucleases are inhibited by the presence of phosphorothioate bonds so as to allow the selected degradation of the unmodified nucleic acid strand. However, any double-strand specific, 5'-3' exonuclease can be used for this process,

provided its activity is affected by the presence of the modified nucleotides. The preferred enzyme when using phosphorothioate derivatives is the T7 gene 6 exonuclease, which shows maximal enzymatic activity in
5 the same buffer used for many DNA dependent polymerase buffers including Taq polymerase. The 5'-3' exonuclease resistant properties of phosphorothioate derivative-containing DNA molecules are discussed, for example, in Kunkel, T.A. et al. (In: Nucleic Acids and Molecular
10 Biology, Vol. 2, 124-135 (Eckstein F. et al., eds.), Springer-Verlag, Berlin, (1988)). The 3'-5' exonuclease resistant properties of phosphorothioate nucleotide containing nucleic acid molecules are disclosed in Putney et al. (Proc. Natl. Acad. Sci. (U.S.A.) 78:7350-7354
15 (1981)) and Gupta A.P. et al. (Nucl. Acids Res., 12:5897-5911 (1984)).

The amplification primers can be used to generate amplified nucleic acid products by any *in vitro* amplification method known to those skilled in the art
20 that uses a DNA dependent or RNA dependent DNA or RNA polymerase. The preferred method is the polymerase chain reaction (PCR) that involves template-dependent extension using thermally stable DNA polymerase (Mullis K et al, Cold Spring Harbor Symp. Quant. Biol. 51:263-273 (1986);
25 Erlich H et al., EP 50,424; EP 84,796, EP 258,017, EP 237-362; Mullis K., EP 201,184; Mullis, K. et al, U.S. Pat. No. 4,683,202; Erlich, H., U.S. Pat. No. 4,582,788; and Saiki, R. et al., U.S. Pat. No. 4,683,194), incorporated herein by reference. PCR achieves the amplification of a
30 specific nucleic acid sequence using two oligonucleotide primers complementary to regions of the sequence to be amplified. Extension products incorporating primers then become templates for subsequent amplification steps. Reviews of the polymerase chain reaction are provided by

Mullis, K.B., (*supra*); Saiki, R.K. et al., (Bio/Technology 3:1008-1012 (1985)); and Mullis, K.B. et al. (Meth. Enzymol. 155:335-350 (1987)), which is incorporated herein by reference.

5 Other nucleic acid amplification procedures can be used and include transcription-based amplification systems (Kwoh, D. et al., Proc. Natl. Acad. Sci. (U.S.A.) 86:1173 (1989)); Gingeras T.R. et al., PCT appl. WO 88/10315 (priority: U.S. patent application Ser. Nos. 10 064,141 and 202,978); Miller, H.I. et al., PCT appl. WO 89/06700 (priority: U.S. patent application Ser. No. 146,462); Davey, C. et al., (European Patent Application Publication No. 329,822)), RNA-dependent RNA amplification (Q β replicase (Kramer, F.R. et al., U.S. 15 Pat. No. 4,786,600), and ligation-based amplification systems (Wu, D.Y. et al., Genomics 4:560 (1989)).

 Amplification primers can be any length but are preferably as long as 90 nucleotides, preferably between 14 to 50 nucleotides, more preferably between 17 and 30 20 nucleotides.

 In accordance with another embodiment of the invention, methods are provided for genotyping a nucleic acid sample comprising hybridizing an invention oligonucleotide to the nucleic acid sample, and 25 preferably, performing a primer extension reaction .

 As used herein, the term "genotyping" refers to determining the presence, absence or identity of a nucleotide base (e.g., a SNP) at a specific position in a target nucleic acid. First, a sample containing the 30 target nucleic acid is treated, if such nucleic acid is double-stranded, so as to obtain unpaired nucleotide

bases spanning the specific position. If the target nucleic acid is single-stranded, this step is not necessary. Second, the sample containing the target nucleic acid is contacted with a genotyping oligonucleotide (also referred to as a genotyping primer) under hybridizing conditions. The genotyping oligonucleotide is capable of hybridizing with a stretch of nucleotide bases present in the target nucleic acid, adjacent the nucleotide base to be identified (e.g., a SNP), so as to form a duplex between the genotyping oligo and the target nucleic acid. When the genotyping oligonucleotide is "immediately adjacent" the nucleotide base to be identified (e.g., a SNP), the genotyping oligonucleotide hybridizes with the target nucleic acid in such a way that either the 3' or 5' end of the genotyping oligonucleotide is complementary to a nucleotide on the target nucleic acid that is located immediately 5' or 3', respectively, of the nucleotide base to be identified (see, e.g., Figure 2A and 2B). It is also contemplated herein that the invention genotyping oligos can be fragments of the oligos in column 3 of the Table 1 (i.e., SEQ ID NO:4n+3, n=0-934) hybridizable to the target nucleic acid and adjacent to the nucleotide base to be identified such that the 3' end of the genotyping oligo is 1 up to 10, preferably 3 up to 6, nucleotides upstream from the nucleotide base to be identified in the target nucleic acid.

As used herein, the phrase "primer extension" refers to enzymatic extension of the genotyping primer in the resultant duplex by one or more nucleotides, catalyzed, for example, by a DNA polymerase, and the like. Such primer extension thus depends on correct base pairing of the added nucleotide to the nucleotide base to be identified.

In one embodiment, the duplex of genotyping primer and target nucleic acid can then be contacted with a reagent containing at least two, but preferably four terminators, at least one of which terminators being
5 labeled. The duplex of genotyping primer and the target nucleic acid is contacted with the reagent under conditions permitting base pairing of a complementary terminator present in the reagent with the nucleotide base to be identified and the occurrence of a
10 template-dependent, primer extension reaction so as to incorporate a terminator at the 3' end of the primer. The net result is that the genotyping primer has been extended by one terminator. Next, the presence or absence of a labeled terminator at the 3' end of the
15 extended genotyping primer is detected. The identity of the labeled terminator indicates which terminator has base paired to the next base in the target nucleic acid. Since the terminator is complementary to the next base in the target nucleic acid, the identity of the next base in
20 the target nucleic acid is thereby determined.

It is also contemplated that the genotyping primer can be extended by a nucleic acid template-dependent ligation reaction. In such a reaction, a ligating oligonucleotide is used that hybridizes to the
25 target nucleic acid only if the nucleotide residue corresponding to the SNP on the target molecule is complementary to the corresponding nucleotide on the ligating oligonucleotide. If such a ligating oligonucleotide hybridizes with the target nucleic acid,
30 the ligating oligonucleotide and the genotyping oligonucleotide will be immediately adjacent each other. The genotyping primer and ligating primer can then be ligated by contacting the target nucleic acid, genotyping

primer and ligating oligonucleotide with a nucleic acid ligase. The ligation reaction can ligate the 5' end of the genotyping primer to the 3' end of the ligating primer, or alternatively, the ligation reaction can
5 ligate the 3' end of the genotyping primer to the 5' end of the ligating primer. Either the genotyping primer or the ligating oligonucleotide can be labeled with a detectable marker. The detection of a ligation product indicates the presence of the SNP nucleotide targeted by
10 the ligation primer.

As used herein, a detectable marker is any molecule or structure that can be detected by spectroscopic, scattering, emission, absorption, binding, or other known detection methods known in the art.

15 Exemplary detectable markers include a radionuclide, a fluorochrome, a colorimetric agent, a magnetic substance, an electron-rich material such as a metal, a luminescent tag, or a detectable binding agent such as biotin. Preferably, the detectable marker is a fluorochrome or a
20 detectable binding agent. More preferably, the detectable marker is a fluorescent dye or a detectable binding agent that can be bound by an antibody. In one embodiment, the detectable marker is biotin or fluorescein. A detectable binding agent refers to any
25 substance that can bind a solid support, a probe molecule, or other molecule that binds the detectable binding agent and, in turn, is then detected or permits identification of the nucleic acid which is labeled with the detectable binding agent. For example, a detectable
30 marker can be biotin which is bound by an antibody specific to biotin, whereupon the antibody can then be detected in an ELISA-based assay. Other detectable markers known in the art may be used in the invention assay, for example, markers listed in the catalog of

Molecular Probes (Eugene, OR), markers listed in the catalog of Synthesgen (Houston, TX), and markers listed in WO 98/59066, which are all herein incorporated by reference.

5 It is further contemplated herein that one or more primer extension reactions, used in genotyping a nucleic acid sample comprising one or more different target nucleic acids, can be carried out in the same reaction vessel. In one embodiment, at least one
10 terminator is labeled with a detectable marker. In another embodiment, one or more genotyping primers are each labeled with a detectable marker. Detection of identity of the SNPs on the one or more target nucleic acids can then be carried out using one of a variety of
15 methods including spectroscopic detection of one or more detectable markers, separation of one or more nucleic acid sequences used to identify the identity of the corresponding one or more SNPs. Such methods for the detection of one or more SNPs in a single reaction vessel
20 are described in WO 98/59066, which is herein incorporated by reference.

 In a preferred embodiment, the primer extension reaction is a "single-nucleotide primer extension" as described, for example in United States Patent No.
25 5,679,524 and in United States Patent No. 5,888,819, incorporated herein by reference in their entirety. In this method, a genotyping primer hybridizes with the target nucleic acid immediately adjacent the nucleotide base to be identified, so as to form a duplex between the
30 genotyping primer and the target nucleic acid such that the nucleotide base to be identified is the first unpaired base in the template target nucleic acid immediately downstream of the 3' end of the genotyping

primer in the duplex of genotyping primer and target nucleic acid. In this embodiment, primer extension will add a single terminator residue which is complementary to the base to be determined in the target nucleic acid.

- 5 Identification of the terminator will thus provide identification of the base on the target nucleic acid to be determined.

Typically, two or more terminators are labeled, each labeled with a different detectable marker, thereby
10 permitting simultaneous detection (and hence, identification) of the two or more labeled terminators. Preferably four terminators are labeled, each with a different detectable marker, thereby permitting simultaneous detection and identification of up to four
15 labeled terminators.

In the embodiment in which only one terminator is labeled (a first labeled terminator), the above-described primer extension procedure can be repeated at least once, using a second labeled terminator that is
20 different from the first labeled terminator. This process can be repeated once, twice or three times, using a different, labeled terminator for each repetition.

As used herein, the phrase "nucleic acid sample" refers to one or more of target nucleic acids,
25 which can be from any source. The sample of target nucleic acids can be natural or synthetic (i.e., synthesized enzymatically in vitro). The sample of target nucleic acids can comprise deoxyribonucleic acids (DNAs), ribonucleic acids (RNAs), or copolymers of
30 deoxyribonucleic acid and ribonucleic acid. The target nucleic acid can be a deoxyribonucleic acid (DNA), a ribonucleic acid (RNA), or a copolymer of

deoxyribonucleic acid and ribonucleic acid. The target nucleic acid can be synthesized enzymatically in vivo, synthesized enzymatically in vitro, or synthesized non-enzymatically. The sample containing the target
5 nucleic acid or acids can comprise genomic DNA from an organism, RNA transcripts thereof, or cDNA prepared from RNA transcripts thereof. The sample containing the target nucleic acid or acids can also comprise
extragenomic DNA from an organism, RNA transcripts
10 thereof, or cDNA prepared from RNA transcripts thereof. Preferably, the target nucleic acid or acids are synthesized by amplification methods described herein. More preferably, the target nucleic acid or acids are synthesized by the polymerase chain reaction.

15 The sample can be taken from any organism, but is preferably human. Some examples of organisms to which the method of the subject invention is applicable include plants, microorganisms, viruses, birds, vertebrates, invertebrates, mammals, humans, horses, dogs, cows, cats,
20 pigs, or sheep. For assay of genomic DNA, virtually any biological tissue samples can be used, including whole blood, semen, saliva, tears, urine, fecal material, sweat, buccal, skin and hair. Genomic DNA is typically amplified by PCR, and the like, before analysis.

25 The target nucleic acid can comprise one or more moieties that permit affinity separation of the target nucleic acid from the unincorporated reagent and/or the primer. The target nucleic acid can comprise biotin which permits affinity separation of the target
30 nucleic acid from the unincorporated reagent and/or the primer via binding of the biotin to streptavidin which is attached to a solid support. The sequence of the target nucleic acid can comprise a DNA sequence that permits

affinity separation of the target nucleic acid from the unincorporated reagent and/or the primer via base pairing to a complementary sequence present in a nucleic acid attached to a solid support. The target nucleic acid can
5 be labeled with a detectable marker; this detectable marker can be different from any detectable marker present in the reagent or attached to the primer.

In a preferred embodiment, the target nucleic acid sequence is synthesized by an amplification method
10 and is modified at the 5' end to prevent hydrolysis by, for example, exonuclease activity, as described herein. See, for example, United States Patent 5,518,900, which describes a method for generating single-stranded nucleic acid molecules containing nuclease-resistant nucleotides.

15 The genotyping primer can comprise one or more moieties that permit affinity separation of the primer from the unincorporated reagent and/or the target nucleic acid. The genotyping primer can comprise biotin which permits affinity separation of the primer from the
20 unincorporated reagent and/or target nucleic acid via binding of the biotin to streptavidin which is attached to a solid support. The sequence of the genotyping primer can comprise a DNA sequence that permits affinity separation of the primer from the unincorporated reagent
25 and/or the target nucleic acid via base pairing to a complementary sequence present in a nucleic acid attached to a solid support.

In another embodiment of the invention, a method is provided for genotyping a nucleic acid sample
30 comprising:

- a) amplifying a target nucleic acid sequence that hybridizes to an oligonucleotide selected from the group of SEQ ID NOs consisting of $4n+3$, or complementary sequences thereof; and
- 5 b) performing a single-nucleotide primer extension reaction employing an oligonucleotide comprising a nucleic acid selected from the group of SEQ ID NOs consisting of $4n+3$, or complementary sequences thereof,
- 10 wherein $n=0$ through 934 and is the same value in both steps a and b.

As used herein, a target nucleic acid sequence that hybridizes to an oligonucleotide selected from the group of SEQ ID NOs consisting of $4n+3$ or complementary

15 sequences thereof refers to a target nucleic acid comprising a sequence substantially similar to at least a portion of nucleic acid of SEQ ID NO: $4n+4$, or complementary sequences thereof that hybridizes to a nucleic acid of SEQ ID NO: $4n+3$. Typically, such a target

20 nucleic acid sequence will comprise the nucleic acid of SEQ ID NO: $4n+4$ with the exception of the nucleotide immediately adjacent the 3' end of the nucleic acid of SEQ ID NO: $4n+3$, which may or may not be present, where n is the same value for both SEQ ID NOs: $4n+3$ and $4n+4$.

25 In a further embodiment of the invention, a kit is provided which contains at least one genotyping primer. As used herein, a kit refers to a genotyping system in kit form, comprising at least one invention genotyping primer, and optionally further comprising

30 chain termination reagent in combination with a DNA polymerase with or without an associated 3' to 5'

exonuclease function, and an appropriate salt and cofactor mixture, in a suitable packaging material. Invention kits are useful for assaying for the presence or absence of a nucleotide at a specified location on one or more target nucleic acids. Preferably, the invention kit is useful for determining the identity of a nucleotide at a specified location on one or more target nucleic acids. To facilitate the assaying for the presence or absence, or determining the identity of the SNP nucleotide on the target nucleic acid, the invention kit provides a genotyping primer, one or more terminators, or both a genotyping primer and one or more terminators, that are labeled with a detectable marker.

The conditions for the occurrence of the template-dependent, primer extension reaction can be created, in part, by the presence of a suitable template-dependent enzyme. Some of the suitable template-dependent enzymes are DNA polymerases. The DNA polymerase can be of several types. The DNA polymerase must, however, be primer and template dependent. For example, *E. coli* DNA polymerase I or the "Klenow fragment" thereof, T4 DNA polymerase, T7 DNA polymerase ("Sequenase"), *Thermus aquaticus* DNA polymerase, or a retroviral reverse transcriptase can be used. RNA polymerases such as T3 or T7 RNA polymerase can also be used in some protocols. Depending upon the polymerase, different solution conditions and different temperature ranges are used for the hybridization and extension reactions.

The reagents of the subject invention typically permit the typing of nucleic acids of interest by facilitating the analysis of the 3' terminal addition of terminators to a specific primer or primers under specific hybridization and primer extension conditions.

Using only one terminator in the chain termination reagent as the nucleoside triphosphate substrate ensures addition of only one nucleotide residue to the 3' terminus of the primer in the polymerase reaction. Using
5 all four terminators simultaneously ensures fidelity, i.e., suppression of misreading.

A genotyping primer of an invention kit comprises a genotyping primer as described above, which is capable of hybridizing to a target nucleic acid. An
10 invention kit comprises one or more genotyping primers. In one embodiment of an invention kit, the genotyping primer(s) comprises any of SEQ ID NO:4n+3, or the complement thereof, where $n=0$ to 934. In another embodiment the genotyping primer(s) comprises a portion
15 of any of SEQ ID NO:4n+4, or the complement thereof, where $n=0$ to 934. In yet another embodiment the genotyping primer(s) hybridizes with any of SEQ ID NO:4n+4, or the complement thereof, where $n=0$ to 934.

A suitable kit includes at least one invention
20 genotyping primer, and optionally further comprising chain termination reagent in combination with a DNA polymerase with or without an associated 3' to 5' exonuclease function, and an appropriate salt and cofactor mixture, as a separately packaged chemical
25 reagent(s) in an amount sufficient for at least one assay. Instructions for use of the packaged reagent are also typically included. Those of skill in the art can readily incorporate invention genotyping primers into kit form in combination with appropriate buffers and
30 solutions for the practice of the invention methods as described herein.

In another embodiment, an invention kit comprises a genotyping primer and two amplification primers. This kit can also comprise chain termination reagent in combination with a DNA polymerase with or without an associated 3' to 5' exonuclease function, and an appropriate salt and cofactor mixture to be used in conjunction with the genotyping primer, and can also comprise nucleotide reagent in combination with a DNA polymerase with or without an associated 3' to 5' exonuclease function, and an appropriate salt and cofactor mixture to be used in conjunction with the amplification primers, in a suitable packaging material. Such invention kits are useful for (1) amplifying one or more target nucleic acid sequences in a nucleic acid sample and (2) assaying for the presence or absence of a nucleotide at a specified location on one or more target nucleic acids. Preferably, invention kits are useful for (1) amplifying one or more target nucleic acid sequences in a nucleic acid sample and (2) determining the identity of a nucleotide at a specified location on one or more target nucleic acids.

Preferably, a kit comprising one genotyping primer and two amplification primers comprises a genotyping primer that hybridizes to the sequence SEQ ID NO:4n+4 and amplification primers of the sequence SEQ ID NOs:4n+1 and 4n+2, where n=0 through 934 and the three oligonucleotides correspond to SEQ ID NOs having the same value for n.

More preferably, a kit comprising one genotyping primer and two amplification primers comprises a genotyping primer of the sequence SEQ ID NO:4n+3 and amplification primers of the sequence SEQ ID NOs:4n+1 and 4n+2, where n=0 through 934 and the three

oligonucleotides correspond to three consecutive SEQ ID NOs having the same value for n.

In yet another embodiment of the invention, a kit comprises two amplification primers. This kit can
5 also comprise nucleotide reagent in combination with a DNA polymerase with or without an associated 3' to 5' exonuclease function, and an appropriate salt and cofactor mixture to be used in conjunction with the amplification primers, in a suitable packaging material.
10 Such invention kits are useful for amplifying one or more target nucleic acid sequences in a nucleic acid sample.

A termination reagent of an invention kit refers to a reagent comprising at least one terminator. Preferably, a termination reagent comprises two
15 terminators. More preferably, a termination reagent comprises three terminators. Most preferably, a termination reagent comprises four terminators. A termination reagent will comprise one or more terminators labeled with a detectable marker. Preferably, a
20 termination reagent comprises two or more terminators labeled with a detectable marker. More preferably, a termination reagent comprises three or more terminators labeled with a detectable marker. Most preferably, a termination reagent comprises four or more terminators
25 labeled with a detectable marker.

A nucleotide reagent of an invention kit for use in an amplification reaction refers to a reagent comprising at least one nucleotide triphosphate. Preferably, a nucleotide reagent comprises two nucleotide
30 triphosphates. More preferably, a nucleotide reagent comprises three nucleotide triphosphates. Most

preferably, a nucleotide reagent comprises four nucleotide triphosphates.

In a particularly preferred embodiment, an invention kit comprises a collection reagents termed

5 "Master Mix" as described in Example I. Exemplary Master Mix 2x is as follows: 768 mL of sterile, distilled water 200 mL of 10xPCR salts (100 mM Tris-HCl, pH 8.5, 500 mM KCl, 15 mM MgCl₂ and 0.01% Gelatin) and 8 mL of each dNTP stock (100 mM stocks of deoxyribonucleotide triphosphates

10 dATP, dCTP, dGTP, and dTTP) in sterile, distilled H₂O. The final composition being: 50 mM Tris-HCl, pH 8.5, 100 mM KCl, 3 mM MgCl₂, 0.002% Gelatin, and 800 μ M each dNTP. This solution can be stored at -20°C until use. Master mix can be prepared in a variety of different

15 concentrations, described as a function of x, as the above example depicts 2x Master Mix. The concentration of the master mix employed herein may vary from 0.5x, 1x, 2x, or 5x, to as high as 10x, 20x, or 50x. Preferably, the master mix is from 2x to 5x.

20 In addition, in place of Tris-HCl, any other buffer which does not interfere with the enzymatic and/or detection processes of required to detect the presence, absence or identity of a SNP may be used. Such buffers include the so-called "Good buffers", and other organo-

25 amino based buffers, and the like. Similarly, the pH may be adjusted to any range capable of carrying out the desired processes. The pH may range from as low as about 6.0, 7.0, or 8.0, to as high as about 8.5, 8.7, or 9.0. Preferably, the pH ranges from about 7.0 to about 8.7.

30 More preferably, the pH ranges from about 8.0 to about 8.5. Buffer concentration may vary from as low as 10 mM, 25 mM or 50 mM, to as high as 75 mM, 100 mM or 200 mM.

Preferably, the buffer concentration is from 25 mM to 100 mM.

In place of KCl, other monovalent-cation salts can be used. Other exemplary monovalent-cation salts include salts with the cation: NH_4^+ , alkalai metal ions Li^+ , Na^+ , Rb^+ , Cs^+ , and other monovalent cations. The anion can be any anion which does not adversely influence the amplification reaction. Exemplary anions are halide anions, F^- , Cl^- , Br^- , I^- , and other anions such as phosphate, sulfate, nitrate, and the like. The concentration of the monovalent salt can be as low as 15 mM, 40 mM, or 75 mM, to as high as 100 mM, 150 mM or 250 mM. Preferably, the monovalent-cation salt concentration is from about 75 to 150 mM.

In place of MgCl_2 , other divalent-cation salts can be used. Other exemplary divalent-cation salts include salts with the cation: Mg^{2+} , Ca^{2+} , Mn^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , and other divalent cations. The anion can be any anion which does not adversely influence the amplification reaction. Exemplary anions are halide anions, F^- , Cl^- , Br^- , I^- , and other anions such as phosphate, sulfate, nitrate, and the like. The concentration of the divalent salt can be as low as 0.5 mM, 1 mM, or 3 mM, to as high as 5 mM, 10 mM or 25 mM. Preferably, the divalent-cation salt concentration is from about 1 mM to 10 mM.

In place of gelatin, other volume exclusion agents can be used such as polyvinyl pyrrolidone, polyethylene glycol, polyacrylamide, linear polyacrylamide, and the like. The volume exclusion reagent can be absent, or can be as low as 0.0001%, 0.0005% or 0.001%, to as high as 0.002%, 0.005% or 0.02%.

Preferably, the volume exclusion agent is about 0.001% to 0.005%.

The concentration of each of dCTP, dGTP, dATP and dTTP (i.e., the dNTPs) can vary in concentration from
5 250 μ M, 500 μ M, or 800 μ M, to 1 mM, 1.5 mM or 2.5 mM.
Preferably, the concentration of the dNTPs is about 500 μ M to 1 mM.

As employed herein, the phrase "packaging material" refers to one or more physical structures used
10 to house the contents of the kit, such as invention primers, and the like. The packaging material is constructed by well known methods, preferably to provide a sterile, contaminant-free environment. The packaging material has a label which indicates that the invention
15 probes can be used for determining the presence or absence of, or preferably the identity of, a particular nucleotide on a target nucleic acid, thereby genotyping the target nucleic acid. In addition, the packaging material contains instructions indicating how the
20 materials within the kit are employed to determine the presence or absence of, or preferably the identity of, a particular nucleotide on a target nucleic acid.

The packaging materials employed herein in relation to diagnostic systems are those customarily
25 utilized in nucleic acid-based assay systems. As used herein, the term "package" refers to a solid matrix or material such as glass, plastic, paper, foil, and the like, capable of holding within fixed limits an isolated nucleic acid, oligonucleotide, or primer of the present
30 invention. Thus, for example, a package can be a glass vial used to contain milligram quantities of a contemplated nucleic acid, oligonucleotide or primer, or

it can be a microtiter plate well to which microgram quantities of a contemplated nucleic acid probe have been operatively affixed.

"Instructions for use" typically include a
5 tangible expression describing the reagent concentration or at least one assay method parameter, such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions, and the like.

10 It is contemplated herein that each of the genotyping and amplification primers described herein can be used in the invention methods of genotyping nucleic acid samples, for example, to assess by association analysis the genotype of an individual, or group of
15 individuals, having a pathological phenotypic trait suspected of being caused by one or more single nucleotide polymorphisms. Phenotypic traits suitable for association analysis include diseases that have known but yet unmapped genetic components, e. g.,
20 agammaglobulinemia, diabetes insipidus, Lesch-Nyhan syndrome, muscular dystrophy, Wiskott-Aldrich syndrome, Fabry's disease, familial hypercholesterolemia, polycystic kidney disease, hereditary spherocytosis, von Willebrand's disease, tuberous sclerosis, hereditary
25 hemorrhagic telangiectasia, familial colonic polyposis, Ehlers-Danlos syndrome, osteogenesis imperfecta, and acute intermittent porphyria, and the like.

Phenotypic traits also include symptoms of, or susceptibility to, multifactorial diseases of which a
30 component is or may be genetic, such as autoimmune diseases, inflammation, cancer, system, diseases of the nervous system and infection by pathogenic

microorganisms. Some examples of autoimmune diseases include rheumatoid arthritis, multiple sclerosis, diabetes (insulindependent and non-independent), systemic lupus erythematosus and Graves disease, and the like.

- 5 Some examples of cancers include cancers of the bladder, brain, breast, colon, esophagus, kidney, oral cavity, ovary, pancreas, prostate, skin, stomach, leukemia, liver, lung, and uterus, and the like.

Phenotypic traits also include characteristics
10 such as longevity, appearance (e. g., baldness, obesity), strength, speed, endurance, fertility, and susceptibility or receptivity to particular drugs or therapeutic treatments.

Such correlations can be exploited in several
15 ways. In the case of a strong correlation between a polymorphic form and a disease for which treatment is available, detection of the polymorphic form set in a human or animal patient may justify immediate
administration of treatment, or at least the institution
20 of regular monitoring of the patient. Detection of a polymorphic form correlated with serious disease in a couple contemplating a family may also be valuable to the couple in their reproductive decisions. For example, the female partner might elect to undergo in vitro
25 fertilization to avoid the possibility of transmitting such a polymorphism from her husband to her offspring. In the case of a weaker, but still statistically significant correlation between a polymorphic set and human disease, immediate therapeutic intervention or
30 monitoring may not be justified. Nevertheless, the patient can be motivated to begin simple life-style changes (e. g., diet, exercise) that can be accomplished at little cost to the patient but confer potential benefits in reducing the risk of conditions to which the

patient may have increased susceptibility by virtue of variant alleles. After determining polymorphic form(s) present in an individual at one or more polymorphic sites, this information can be used in a number of
5 methods.

In addition, determination of which polymorphic forms occupy a set of polymorphic sites in an individual identifies a set of polymorphic forms that distinguishes the individual. See generally, National Research
10 Council, The Evaluation of Forensic DNA Evidence (Eds. Pollard et al., National Academy Press, DC, 1996). Thus, each of the genotyping and amplification primers described herein can be used in the invention methods of genotyping nucleic acid samples, for example, to identify
15 a distinguishing or unique set of forensic markers in an individual useful for forensic analysis. For example, one can determine whether a blood sample from a suspect matches a blood or other tissue sample from a crime scene by determining whether the set of polymorphic forms
20 occupying selected polymorphic sites is the same in the suspect and the sample. If the set of polymorphic markers does not match between a suspect and a sample, it can be concluded (barring experimental error) that the suspect was not the source of the sample. If the set of
25 markers does match, one can conclude that the DNA from the suspect is consistent with that found at the crime scene. If frequencies of the polymorphic forms at the loci tested have been determined (e. g., by analysis of a suitable population of individuals), one can perform a
30 statistical analysis to determine the probability that a match of suspect and crime scene sample would occur by chance (see, e.g, WO 95/12607). If several polymorphic loci are tested, the cumulative probability of non-identity for random individuals becomes very high (e. g.,

one billion to one). Such probabilities can be taken into account together with other evidence in determining the guilt or innocence of the suspect.

In addition, each of the genotyping and
5 amplification primers described herein can be used in the invention methods of genotyping nucleic acid samples, for example, to identify a distinguishing or unique set of markers in an individual useful for paternity analysis. The object of paternity testing is usually to determine
10 whether a male is the father of a child. In most cases, the mother of the child is known and thus, the mother's contribution to the child's genotype can be traced. Paternity testing investigates whether the part of the child's genotype not attributable to the mother is
15 consistent with that of the putative father. Paternity testing can be performed by analyzing sets of polymorphisms in the putative father and the child. If the set of polymorphisms in the child attributable to the father does not match the putative father, it can be
20 concluded, barring experimental error, that the putative father is not the real father. If the set of polymorphisms in the child attributable to the father does match the set of polymorphisms of the putative father, a statistical calculation can be performed to
25 determine the probability of coincidental match (see, e.g., WO 95/12607). If several polymorphic loci are included in the analysis, the cumulative probability of exclusion of a random male is very high. This probability can be taken into account in assessing the
30 liability of a putative father whose polymorphic marker set matches the child's polymorphic marker set attributable to his/her father.

The single nucleotide polymorphisms set forth in column 4 of Table 1 (also referred to herein as SEQ ID NO:4n+4, wherein n=0 through 934) may contribute to the phenotype of an organism in different ways. Some of these polymorphisms may occur within a protein coding sequence and contribute to phenotype by affecting protein structure. The effect may be neutral, beneficial or detrimental, or both beneficial and detrimental, depending on the circumstances. For example, a heterozygous sickle cell mutation confers resistance to malaria, but a homozygous sickle cell mutation is usually lethal. Others of these polymorphisms may occur in noncoding regions but may exert phenotypic effects indirectly via influence on replication, transcription, and translation. A single polymorphism may affect more than one phenotypic trait. Likewise, a single phenotypic trait may be affected by polymorphisms in different genes. Further, some of these polymorphisms may predispose an individual to a distinct mutation that is causally related to a certain phenotype.

Phenotypic traits include diseases that have known but yet unmapped genetic components. Phenotypic traits also include symptoms of, or susceptibility to, multifactorial diseases of which a component is or may be genetic, such as autoimmune diseases, inflammation, cancer, diseases of the nervous system, and infection by pathogenic microorganisms. Some examples of autoimmune diseases include rheumatoid arthritis, multiple sclerosis, diabetes (insulin-dependent and non-independent), systemic lupus erythematosus and Graves disease. Some examples of cancers include cancers of the bladder, brain, breast, colon, esophagus, kidney, leukemia, liver, lung, oral cavity, ovary, pancreas, prostate, skin, stomach and uterus. Phenotypic traits

also include characteristics such as longevity, appearance (e. g., baldness, obesity), strength, speed, endurance, fertility, and susceptibility or receptivity to particular drugs or therapeutic treatments.

5 Correlation is performed for a population of individuals who have been tested for the presence or absence of a phenotypic trait of interest and for polymorphic markers sets. To perform such analysis, the presence or absence of a single or a set of polymorphisms
10 (i. e. a polymorphic set) is determined for a set of the individuals, some of whom exhibit a particular trait, and some of which exhibit lack of the trait. The alleles of each polymorphism of the set are then reviewed to determine whether the presence or absence of a particular
15 allele is associated with the trait of interest. Correlation can be performed by standard statistical methods such as a chi-squared test and statistically significant correlations between polymorphic form(s) and phenotypic characteristics are noted. For example, it
20 might be found that the presence of allele A1 at polymorphism A correlates with heart disease. Such correlations can be exploited in several ways as set forth above.

 For example, in the case of a strong
25 correlation between a set of one or more polymorphic forms and a disease for which treatment is available, detection of the polymorphic form set in a human or animal patient may justify immediate administration of treatment, or at least the institution of regular
30 monitoring of the patient. Thus, the methods of genotyping nucleic acid samples described herein can be used, for example, to diagnose any of more than 3000 genetic diseases currently known or to be identified,

e.g., hemophilias, thalassemias, Duchene Muscular Dystrophy (DMD), Huntington's Disease (HD), Alzheimer's Disease and Cystic Fibrosis (CF), and the like.

It is also contemplated herein that each of the
5 genotyping and amplification primers described herein can be used in the invention methods of genotyping nucleic acid samples, for example, for assessing the pharmacogenomic susceptibility of a subject harboring a single nucleotide polymorphism to a particular
10 pharmaceutical compound, or to a class of such compounds. Genetic polymorphism in drug metabolizing enzymes, drug transporters, receptors for pharmaceutical agents, and other drug targets have been correlated with individual differences based on distinction in the efficacy and
15 toxicity of the pharmaceutical agent administered to a subject. Pharmacogenomic characterization of a subjects susceptibility to a drug enhances the ability to tailor a dosing regimen to the particular genetic constitution of the subject, thereby enhancing and optimizing the
20 therapeutic effectiveness of the therapy.

All U.S. patents and all publications mentioned herein are incorporated in their entirety by reference thereto. The invention will now be described in greater detail by reference to the following non-limiting
25 examples.

Examples

Example I

Preparation of 2x Master Mix for PCR

A component of genotyping using SNPE is
5 accurate and efficient amplification of the nucleic acid
region being tested for the SNP. To facilitate control
of the PCR used, a reaction mixture, referred to herein
as "2x Master Mix," has been produced to achieve more
successful PCRs.

10 2x Master Mix was prepared as follows: 10xPCR
salts was 100 mM Tris-HCl, pH 8.5, 500 mM KCl, 15 mM MgCl₂
and 0.01% Gelatin, stored in a -20°C freezer. 100 mM
stocks of deoxyribonucleotide triphosphates dATP, dCTP,
dGTP, and dTTP (dNTPs) (LTI, Bethesda, MD) were made in
15 sterile, distilled H₂O. To 768 mL of sterile, distilled
water 200 mL of 10xPCR salts and 8 mL of each dNTP stock
was added. The final composition was: 50 mM Tris-HCl, pH
8.5, 100 mM KCl, 3 mM MgCl₂, 0.002% Gelatin, and 800 µM
each dNTP. The solution was stored at -20°C until use.

20

Example II

Single Nucleotide Polymorphism Detection by Single Nucleotide Primer Extension (SNPE)

Single nucleotide polymorphisms are the most
25 common form of genetic diversity and are thought to be
direct and indirect markers of many human diseases. The
following example shows the use of Single Nucleotide

Primer Extension (SNPE) to identify the presence of SNPs. The method of SNPE is the subject of US Patent 5,888,819 and more recently described in detail by Reynolds et al. in *DNA Markers: Protocols, Applications, and Overviews* (ed. G. Caetano-Anolles), pp. 199-211, Wiley-Liss, New York, NY (1997), both of which are herein incorporated by reference.

The amplification primers listed herein (Table 1, columns 1 and 2) were selected to amplify the corresponding SNP-containing oligo in column 4, forming a PCR amplified target nucleic acid such that the primers do not overlap the corresponding genotyping (SNPE) primer in column 3.

SNPE primers were designed to be complementary to the phosphorothioate-modified strand of the PCR product target nucleic acid and ending one nucleotide base short 3' to the polymorphic site to be interrogated as described (Reynolds et al., *supra*). Either DNA strand can be a target for the SNPE primer as long as the phosphorothioate-modified strand of the PCR product is the complementary strand.

All oligonucleotides were synthesized by standard phosphoramidite chemistry on a PE Biosystems 392/394 DNA Synthesizer using Glen Research (Sterling, VA) reagents. Tetraethylthiuram sulfide (TETD; PE Biosystems, Foster City, CA) was used for phosphorothioate modification as per manufacturer's instructions. All oligonucleotides were deprotected in concentrated ammonia and desalted using NAP5 or NAP25 gel filtration columns (Amersham Pharmacia Biotech, Piscataway, NJ) as per manufacturer's instructions.

Genomic DNA from genetically diverse human individuals purchased from the Coriell Institute for Medical Research (Camden, NJ) was the source of DNA for PCR amplifications. The DNA sample was diluted with
5 sterile, distilled H₂O to a final concentration of 5 ng/μL before use.

PCR amplification of 10 ng of template genomic DNA was performed in 384-well plates under the following conditions: 10 mM Tris-HCl, pH 8.5, 50 mM KCl, 1.5 mM
10 MgCl₂, 0.001% gelatin, 400 μM each of dATP, dCTP, dGTP, and dTTP, (from the 2x Master Mix) and 0.050 U/μL Platinum Taq (*Thermus aquaticus*) DNA polymerase, (LTI, Bethesda, MD) using 0.5 μM each primer concentration in a total volume of 5 μL. The PCR amplification was
15 performed in a PTC-225 DNA Engine Tetrad thermocycler (MJ Research, Watertown, MA) using the following protocol: following a 2 minute denaturation step at 95°C thirty-five cycles were carried out, each consisting of denaturation (30 seconds at 94°C), annealing (2 minutes
20 at 55°C), and extension (30 seconds at 72°C) steps. This was followed by a final extension step (1 min at 72°C) and hold (4°C).

Single stranded target nucleic acid DNA was prepared from the double-stranded PCR product by
25 treatment with a 5'-3' DNA exonuclease. Briefly, 5 μL T7 gene 6 exonuclease (United States Biologicals, Swampscott, MA) in 0.5 M Tris-HCl, pH 7.5, 1 mM Dithiothreitol (DTT), and 0.01% acetylated Bovine Serum Albumin (BSA) was added to a final concentration of 0.45
30 U/μL and incubated for one hour at room temperature. The reaction was terminated by the addition of 5 μL of 3 x SNPE Salt (4.5M NaCl, 30 mM EDTA, and 3 mM cetyltrimethylammonium bromide (CTAB)). The DNA created

by PCR originating from the phosphorothioated primer is protected from nuclease digestion whereas the opposite strand with the unmodified primer is digested and hence will not interfere with the hybridizing strand to the
5 immobilized genotyping (SNPE) primer.

The SNPE primer of interest was attached to a polystyrene 384-well plate (NUNC). Briefly, 20 μ L aliquots of 0.25 μ M SNPE oligonucleotide in 50 mM N,N-dimethyloctylamine hydrochloride, pH 7.0 (ODA; Segma-
10 Aldrich, Milwaukee, WI) was added to each well and incubated overnight at 37°C. The plates were washed with TNTw (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween-20) three times and once with 1xTE/ISOH (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10% isopropanol). The plates were
15 incubated upside down at 50°C for 30 minutes prior to storage in sealed pouch with dessicant. 15 μ L of the exonuclease digestion product was added to the appropriate well and the single-stranded PCR product was allowed to hybridize for 30 minutes at room temperature.
20 All wells were subsequently washed with TNTw three times.

An exonuclease (-) version of the Klenow Fragment of *E. coli* DNA Polymerase I (United States Biologicals, Swampscott, MA) was used for template-directed single nucleotide extension of the SNPE primer.
25 Briefly, the enzyme was diluted in a buffer containing 10 mM Tris-HCl, pH 7.5, 5 mM DTT and 0.5 mg/mL BSA. The extension reaction was performed in 20mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 25mM NaCl, 10 mM MnCl₂, 15 mM Sodium Isocitrate, 1.5 μ M each of the four 2', 3'-
30 dideoxynucleoside 5'-triphosphates (ddNTPs), ddATP, ddCTP, ddTTP, ddGTP. Two of the ddNTPs were unlabeled, one was labeled with biotin, e.g. 2', 3'-dideoxyuridine 5'-triphosphate (biotin-ddUTP), and one labeled with

fluorescein, e.g. fluorescein-labeled 2', 3'-
dideoxycytosine 5'-triphosphate (fluoresceinated-ddCTP).
The determination of which labeled ddNTPs to use depends
on the nucleotide identity of the presumed SNP. For
5 example, a suspected C to T mutation can be assayed using
a mix of ddATP, fluorescein-ddCTP, ddGTP, and biotin-
ddUTP. The enzyme concentration was 0.02 U/ μ L and the
reaction was performed at room temperature for 30
minutes. The plates were washed with TNTw three times,
10 then 0.2N NaOH, and then three times with TNTw.

Detection of the extended primers was performed
by standard ELISA techniques using two nucleotide-
specific labeled antibody conjugates. The dilutions of
each are determined empirically for each antibody lot to
15 achieve approximately equal intensity for the two
colorimetric signals in the presence of both alleles.
Briefly, the wells were incubated for 30 minutes at room
temperature with 10 μ L of 1% fraction V BSA (Sigma, St.
Louis, MO) in TNTw containing an alkaline phosphatase
20 conjugate of antifuorescein (1:2000 dilution)
(Boehringer Mannheim, Indianapolis, IN). After washing
with TNTw six times, the presence of alkaline phosphatase
was determined first by the addition of 25 μ L per well of
a 1.5 mg/mL solution of p-nitrophenyl phosphate (Moss,
25 Pasadena, MD) in 100 mM diethanolamine, pH 9.5, 20 mM
MgCl₂. The plate was immediately placed in a microplate
reader (ICN, Costa Mesa, CA) and the development of color
monitored spectrophotometrically at a wavelength of 405
nm after 24 minutes in an endpoint assay. Next, the
30 detection of biotinylated-ddNTP was performed. The wells
were incubated for 30 minutes at room temperature with 10
 μ L of 1% fraction V BSA in TNTw containing a horseradish
peroxidase-conjugated antibiotin (1:500 dilution) (Zymed,
San Francisco, CA). The plates were then washed three

times with TNTw and the presence of fluorescein was determined by the addition of 25 μ L of a 1 mg/ml solution of tetramethylbenzidine (TMB; Moss, Pasadena, MD). The plate was immediately placed in a microplate reader and the development of color monitored spectrophotometrically at a wavelength of 620 nm after 24 minutes in an endpoint assay.

A scatterplot of the raw data of OD₄₀₅ v. OD₆₂₀ was used in the visual determination of sample genotypes. A polymorphism is identified when three distinct clusters are seen in the positive regions of the scatterplot. These correspond to individuals that are homozygous, forming one cluster that is positive for OD₄₀₅ and another that is positive for OD₆₂₀, or heterozygous, forming a third cluster that is positive for OD₄₀₅ and OD₆₂₀.

Figure 3 shows a scatterplot that was classified polymorphic. The PCR primers used were oligonucleotides of SEQ ID NOS:25 and 26 (Ref No. 261, Table 1). The SNPE primer used was an oligonucleotide with SEQ ID NO:27. The targeted nucleic acid with the suspected polymorphism contains the sequence of SEQ ID NO:28. SNPE was performed using fluorescein-ddCTP and biotin-ddUTP. The Y-axis corresponds to signal obtained from ddUTP incorporation and the X-axis corresponds to signal obtained from ddCTP incorporation. Note that there are clusters where each allele tested is positive (CC or TT) and where both alleles are positive (CT). This is an example where a SNP is identified. Note that there is another cluster about the origin which corresponds to no signal (NS) due to PCR failure.

Table 1 lists the invention oligonucleotides disclosed herein. Each row provides two PCR primers used

to amplify the target DNA comprising a certain SNP
(Columns 1 and 2), one genotyping oligonucleotide (Column
3; referred to as a SNPE primer) used to probe the
identity of the base at the location in the sequence
5 corresponding to that SNP), and sequence of the region
flanking the site of that SNP (Column 4). A "P" listed
to the right of a PCR primer denotes a phosphorothioation
of that primer at its 5' end. In this embodiment, such a
phosphorothioation refers to the five most 5' nucleotides
10 of one of the two PCR primers being linked by four
exonuclease-resistant phosphorothioated linkages (e.g.,
5' C-(p)-G-(p)-C-(p)-A-(p)-GTCTCAGGCCAGCT 3', for SEQ ID
NO:2, where "-(p)-" represents a phosphorothioate
linkage). While phosphorothioation sites are listed for
15 the specific residues herein, it is understood that other
modifications which confer exonuclease resistance can
serve as equally desirable substitutes.

In this particular embodiment, nucleotides
labeled as "X" refer to C3 linkers placed in the
20 corresponding sequences at the location of the X. While
C3 linkers are listed herein, it is understood that other
single-nucleotide-spacers can serve as equally desirable
substitutes.

The sequences of Table 1 are arranged in the
25 same manner as the SEQ ID NOs are listed. Accordingly,
the nucleic acids of SEQ ID NOs:1, 2, 3 and 4 correspond
to the nucleic acids in Row 1, Columns 1, 2, 3 and 4,
respectively. Similarly, the nucleic acids of SEQ ID
NOs:5, 6, 7 and 8 correspond to the nucleic acids in Row

2, Columns 1, 2, 3 and 4, respectively. Therefore, a general formula can be established to describe the SEQ ID NO of each nucleic acid:

$$\text{SEQ ID NO} = (4 \times (\text{Row Number} - 1)) + \text{Column Number}$$

Row#	REF#	Upper PCR primer	1	2	Lower PCR primer	3	Modified cBA primer	Flanking sequence
1	255	TTGAAATGTGAATAGTAATAACTATGTAGAGATGA			GGCAGTCTCAGGCGAGCT	P	CAGTATGGGAAAAAGACAGCTGGGGTCTCGGAG	GTATGGGAAAAAGACAGCTGGGGTCTCGGAGAGAGATGA
2	256	CTCTTGGCTAGTTTATGTGAGATGAGA			AGCTTTCAATCCTTCCTCAACTTG	P	TCTACTTTCATCTCACTTCTCTTATTA	TCTACTTTCATCTCACTTCTCTTATTAACGACTAAATCTCTCTCATGTGTTTACCA
3	257	CTACTGAGCTCTTGACTGAGCTAGCTAATTAAT			AATACATGAAAGCCCAAAATACACA	P	AATGACCTCTCTGTCATATCTAGCTGAGGGATAGCTCTCAGAGACGAT	GCTTGCCCTCTCTGTCATATCTAGCTGAGGGGATAGCTCTCAGAGACGAT
4	258	ATATATGCTTTCTGTGAGGTGAAG			TCAAAGGAAAGGTATGTGCAATATT	P	AAGTGCATCGCCCTCTCAGAAACGAGG	ATTTTGTCTGTAGTAATCTGTGTCACACAGCTCTGAGAGCCGATGCACTT
5	259	ATCTCAATTCACGCTTTGCTCT			CATGAGGCGAAATTCCTTAACCTTT	P	CTTTCCGACAGAGGTCTCCCTGAGCG	AGCGGGAGCCTCAGCTCTCACTGCTCTCTCTCGAGGACCTCTGTTGCGAAG
6	260	AAGCAGCTCTGACTTTTGAGT			CCCTCTGCACCACTTAACCTTAAGT	P	GTGTTTCACTAATAAGTACAGCAATTTTT	CTGACTTCAAGACGTCATTTGATCTTAAAAATGTCTGCACTTATGTATGTGCAAC
7	261	GTGGATATGATTAATTCAGACATGGACATTA			ATTATTTGTTTTGGCTCTCGATT	P	ATTATTTGAGAAATXCHAGATCATAGA	TTATTTCAAGAAATCAGATGATACAGACAGAGACTCTGAGCTCAGTACGACTGAGA
8	262	CGAGCATCAGGTTATTGAAGC			ATTAGGAGCTAGCCCCGCT	P	CAGAGGCCCTGTCAAGAGCTACCC	CAGAGGCCCTGTCAAGAGCTACCCGTGATGCAAGTCTGAGCTGAGGAGTAGA
9	263	CAAGTCACTAAGGGGCGTTG			CTCAGCTCAGCCCTTCTCCATC	P	TTAGAACACTGGGGGATTTACCTTC	TTAGAACACTGGGGGATTTACCTTGCACTTTTAAAAATAGACTCATGAT
10	265	TGAGGAGAGGGCGTCA			GAAATCTGTTTTTCCCAAGAGA	P	CAAGAGGAGTCTCTCTTTTCTCATCC	CAAAATCTTTGAGTGTCTGATTTTGGGAGTAGAAAGAGGAGGCCCTCCCT
11	266	GTGCTGGAGTCCGGACATGATTA			ACNCCAAAGGTTTTTGTGATATC	P	AATGAGTAGGGGCTATTAATGAAATTTA	AATAACTTATTTTGCAAAATGTGTACTAANAATTTTAAATAGCCCTTATCTCA
12	267	TATTTTCCCAACCTTTTGG			GAIAAAACCAAGAGGGTGC	P	AATACCACTATTTGATTTCTCTGTA	AAATGTGTTCTGATGTGAATTTAAAGTGTCTGAGTTTGGGGGCTTCTCTG
13	269	AGTGAAGGATCRGTCAKGC			CATATAAATCAGGAGCAGAGACAGAGA	P	CAGAGAGGCCCCCAAACTCAGACA	AATGACATATGATCTTAAGAGCTGGCAAAATTTGCTGTATGTGTA
14	270	AGGATCRGTCAKGCATTAAGTTAG			AAACTCAGCAATTTAAATTTCAATCAGA	P	TTCACTCAGAAACATTTTGGCCACT	AACTGACATATGATCTTAAGAGCTGGCAAAATTTGCTGTATGTGTA
15	271	ATGGTTTAAAGTGTGTGGAGCTTC			CATGCAGACAGTGGAGG	P	ACTTTTGTCACTCTCTCTCTCTG	ACGTTTGTCACTCTCTCTCTCCGACCACTGCTGAATTTGATGTGCTT
16	272	TGACTGGCAGATTCACAAAGAA			CTATGGCCCTGCATGGCA	P	AGTCTCTAAATGATGAGGGTGAAGC	TCTCTTAATGATGAGGTGAAAGGTGGGAGTCTGCTGTAAAGGCCCAT
17	273	TGTACTGCTCTTCTCATATGCTGAT			GTTACTGATCAGGGGTGATATAG	P	ATAGATGATGATGATGATAGATGA	CATTCACTGTCTTTCTATCTCTCCGTCCATCTATCCATCCATCATCTAT
18	274	AACTTGATTTTCTGATGTGACCTTATATT			TCATTTACTATTTATGACCTGGATACTTCTATAA	P	TTCTATTAGACXAATTTTACAGTTGAT	CATTTAGAGTAAATTTTACGTTTATGAGGAGTGGGGGATTTTATTTTATAGAGAT
19	275	AAATTTGCTGGGACACA			CTAAAGATTCGAGCAGATGACGCC	P	ATGCAACCGCTCCACAAACAACAAA	ACGCTTCACTTAACCAATGTGTGAGTTTGTGTTTGTGAGGGTTTTGCAT
20	276	GTGGAACAGATAGACAGACAGAA			TGCGAGTGTGTGCCAATAGTTCT	P	TTGTTCCATGTATTTGTGCCCTTTTC	CAATTTAGAGTAAATTTTACGTTTATGAGGAGTGGGGGATTTTATTTTATAGAGAT
21	277	ATATATATCATTCAGCAGATATTATGCAATG			CTGTGATCAGGGCTGCTT	P	TTGGTGGCCCACTAGCCCTTTGCCCTT	TAATTAAGTAATAAAGAGGCTGTGATCAAAGGAGAGGCTAGTGTGGGACCA
22	278	TGCTATGATCTTAACCTTCCCTGTACC			CAATGAAATGGCTATGAAAGTCT	P	GTGCTGATGATGACACTTCTGGGGT	CTCTGGATTTTGAGGTTTGCATGGGTCACGCCACGAGTGGCATCATAGAG
23	279	GGGTATTACATTTGCTCTTAATCATAT			TGGGAATAGATGACGCCACA	P	ATTGCAATTTGTTTAAATTAATTTTGTCTGTCTGAGCAGTAAATTTCTCA	CAGCATATGTTGTTTAAATTAATTTTGTCTGTCTGAGCAGTAAATTTCTCA
24	280	GATCCTACTTTTGGGACCGGT			CCAGGAGTGGAGAGATGCG	P	GAATTTTTTAAATGATATATACACTATA	TCATTTGTACCCCTCCCATCGAACCGAGTATAGTCTATAATCAATAAAAAAT
25	281	CTAGTTCTTTTGGAAATTAAGCCAA			TCTTCAACCTTATTTTGACAAATTC	P	CATGACTCTTAAGTGGATCAAAAGTGTGTCAGATGTCTGCATGAAATTTG	CATGACTCTTAAGTGGATCAAAAGTGTGTCAGATGTCTGCATGAAATTTG
26	282	ATTAGTGCCTCAGTAATACTAGTGG			CTTGGCCCTCTCAGGCG	P	TGGGAGCTGAGAGACACAGAGAAACCAAGATATTTTCTAGCGCTTAAT	TGGGAGCTGAGAGACACAGAGAAACCAAGATATTTTCTAGCGCTTAAT
27	283	TGGGGTACAGAAAGTTTACONTTACA			ACACCTGCACCTCCAGCCGTG	P	AAAGTGTGACTCCGCTCTCAAAAAA	AAAGATGTTTTATTTGTTTTTTTTTTTTTTTTTTTGTAGACGGAGTGCACACTT
28	284	TGTGACTGCTTAATCAGTTTCTGAG			CCAGATGCAATTTCTGCAAC	P	CAGAGAGACTTGAAGTCTTGGAACTC	TCGAGACTAGAGTGTCTCCAGCAGAGATGTCCCAAGACTCTCMAGTCTCTCG
29	285	AGCAACCAAAAAAATAATGTGTAGA			CTAAGAAATGGAAATCTCTGCCCT	P	AGCAAAAGCAAGGATATTTGTCTGGGTAGAGCCGTGGGGCCCACTAGGGG	AGCAAAAGCAAGGATATTTGTCTGGGTAGAGCCGTGGGGCCCACTAGGGG
30	286	CTGGCGCAGGCGTAAACA			CAAAATGGAAATTTTCAATGTGTTCCC	P	TTGTTGCTTCAAGCAAGAACTTACGGCTGGGTCAAAATTTCAAGAGACTCTG	TTGTTGCTTCAAGCAAGAACTTACGGCTGGGTCAAAATTTCAAGAGACTCTG
31	287	CAATTTCAATGTGCTAAAAACGAG			TTTTTGGAAATTTTTCATGATGTTCCC	P	TGTTCCCAACTCTGACVACTCATAGG	ATCAAGTAAACCTGTGAACAAATAGCCTATAGTGTCAAGTTTGGGNAACA
32	288	TGAGCAGTATTTGTAGGC			TGCTATAAGACATACCCAGACCTGG	P	ATGCGAACTGTGAGTCAATTAACACTT	GTGCAACTGTGAGTCAATTAACACTCTTTCTTTCTATAAATTAACCCAGTCTT
33	289	CTCACCAACTACACTTGTGRTATTAGC			GTAATTTCTCATCTAAATGAAAGCAATTTT	P	AAAGCACAATTTTATAGAGAGAAAGA	ACTTTTATAATTTCTATATCTTACAGCTCTTCTCTCTCTTAANAATGTGCTTTT
34	290	CAAAAAATACAGCAATTAAGACAGAC			ATATGTTGGGTGGCAGCTTAGC	P	CTATAGATA CAGATATGATATTTATTA	CTATAGATA CAGATATGATATTTATTAACGGGAATTTGGGCTCATGCTATATAGA
35	291	ACTCATGATATACAGGCTCTT			GAAAGTTGGAGGTACAGGTCT	P	TCTGGTTGCACGXCXCATCCCTGGGCA	TGGTTGCACCCCATCCCTGGGCGAGTGAAGAAATTCATGAGGCCCATCTCT
36	292	GCTACATCTTCTTGTGCTATCATTT			TCAATGTATTCATGCAATGTGTGATG	P	AATGXCXAAATGTGATGATGTGTGTT	AAAGATGCTCTTTCATTAACCAACAGCAAGCTTACCATCATCTTTTGAAGA
37	293	GGGCTCAGCAAAATACAGGT			CCGGGATCTCGAGTGACA	P	TGGTAAANAAGATTTTCAATGTGATTA	TCAACAGACATCAAAAAATTTTGAATTAACACATCAAAATCAATTTTTTTAC
38	294	ATTCAGTCCAGGCTGGG			GCATCTAGCATGAAACATAGC	P	ATAGCTGGGAAGACACCTCCAGCCGGAGCTCAATGATGGCTCTTTCTAT	ATAGCTGGGAAGACACCTCCAGCCGGAGCTCAATGATGGCTCTTTCTAT
39	295	CTTCAAGCCTCCACTACATCCTC			AGTAGGAGAAACATGAGAGTTTTTTTAA	P	TCCAAATTTCTTGATAAACATGCAAGCTATTTTTTCTGAGAAAATAGAGCG	TCCAAATTTCTTGATAAACATGCAAGCTATTTTTTCTGAGAAAATAGAGCG
40	296	CATGGGAGAGAGATCTAGTAAGTG			TCACAGAGGAGTTCAGATATAAAA	P	GGAAATTCATCTXTATAGACATATTA	GGAAAGTCACAAAATTCGAAGAAACCTTAATGAATATGCTCTATAGATGATTTTC
41	297	GCATCTTTTGAGATTTCGATC			AGAGGCAATGATCAGTCCAGG	P	AAACCCCTCTATCTCTGAGTGTGTC	AAACCCCTCTATCTCTGAGTGTGTGCCCAAAATAGAAAAAATGTTTTAT
42	298	CTACAGCAGCCTCAGACCC			GTATACAGGCGAGGTAGAGAAAGG	P	CAGACTTTTAAAGXATGTTGCTCCATTA	GAIAAAATGTTTTTATAATGAGCAGCATATGAGGCAACATGCTTAAAGGCTC
43	299	AGCAGCTAAACCCCTCTATCC			CTGGGAATGGAAGAAACGACA	P	GGAGAGAGTGGCCCTTATAGCAACANT	AGCTTATCTCCACTTTTCTGGCCTGATGTCTGTCTTAATAGGGCCCATCTCTG
44	300	TACAGGGATTTTGGATCTTTCT			ATTTATCTCTCAGAAACATCTCTACT	P	AGATCCAGCTTAGAATCCCATTTCCA	AGATCCAGCTTAGAATCCCATTTCCCACTGTGTCTGAGTATCTCTTTAT
45	301	TTGATCTTACTCAGCTCTCTCAGAG			TTTTAAGATTTTTCAGGGCAGGTTTC	P	CAGGTTCTGAATAGCCCTTTGCTGTA	TCTGAGTCTGAGGGCTAAATATGAGCGTAGACCAAGGCTATTTCAGAGACCTG
46	302	TTAATTCGAGACACTCTTTATATATCGG			GAAATGACTTGGCTAGGCCA	P	AAACCKXTTACAGACAAAGATTTCTG	CCCTTTAGCAAGACAAAGATTTCTGAGACGCTGAGCTCTCCCATCTAGCTAGCGCG
47	303	AATAGAAACAAAGGAAGTTTCAATCTCG				P	CTATCTCCACCGCAGAGTGGACTTGGT	ATTTTAAATGAGTATTCATGCTCAATGCTCTCTCTCTGCGCAATTCGAGCGCAATGMA
48	304	AATTGAGCATCTAGACAGACCCCC			GTTTGATCTCCAGATGTGTAAGAGATG	P	ATTTTAAATGAGTATTCATGCTCAATA	ATTTTAAATGAGTATTCATGCTCAATGCTCTCTCTCTGCGCAATTCGAGCGCAATGMA
49	305	CTCTGATCTGTGGCAATTAATAGTAAGA			CTTGAATAACACATCTTGCCTTAGTTGCT	P	ATTTTAAATGAGTATTCATGCTCAATA	ATTTTAAATGAGTATTCATGCTCAATGCTCTCTCTCTGCGCAATTCGAGCGCAATGMA

Row#	REF#	Upper PCR primer	1	Modified	Lower PCR primer	2	3	Planking sequence
50	306	TAAGTGGGCACTGAGCATG	P		GAATGTGGCRITTTATTACATAGGTATACA	P	CCACXGTGGTTTGGGGXTCACCTCTTT	AGCCACCTTAAGAGAGAGAAATCATGGGAAAGAGGTGATCCCCAAACCAACCGT
51	307	TACCAGAAAAGTGGACATCTCT	P		CTTCTGAGCGCCTCCAAACTGT	P	TGAGATACCTCGAATAATCTGGAAGC	TGAAGATACCTCGAATAATGTCGAAGCAGCTTTTAAAAATTTGGGTGAAGCAGCAGA
52	308	TTTGATGTTTGTGGACACCTGT	P		GTATGATTAAGCAATTTTATATGCTTTC	P	TGGCTTGTGGCTTGTGACCTAGCT	TTCCACAGGTTTGGCTGTTTGACCAACAGCAGAGGTCAAAAGCCACAGAGCCGA
53	310	AAATATACAGACAGCTGTTTACTGGTG	P		CTATGCGGATATAGGCATGAGC	P	GGGAGGTAGACAGAGCATACAATA	GGGAGGTAGACAGAGCATACAAGTACAGAGTCAAAAGCCACAGCTGTGGCTC
54	311	GTGGACCTAAATTAACCTAAMAGCTTC	P		TGTCTGATGCGACAGTTTGAATAATT	P	CAAAATAACTACCAACAGAGTAAA	CAAAATAACTACCAACAGAGTAAACAGACATCAACAGCACCACAGATAGGAGGAAA
55	312	TTTTCACTCATATGCGAAGCTAAAC	P		CAATTAACCTTCTCTCTCTCTCC	P	CTCTCCCTCTCCCAACACCTCTTC	CTAGATTCAATGTTACCAGAGCTTCCGAGAGCATGGTCGGGAGAGGAGCAGAC
56	313	GGCCACACATGATTTCTTACATG	P		TBATCTGTACACATCTCTGGGTTC	P	TGCTGGAAATGCAATTTTGTGTATATA	ATATGAGAAAAATTTTAAATCAATTTGTGATATATACAAAATCAATTCACAGGCA
57	314	GCAGATGCCCTCTTACTAGTACT	P		GTGAAATAGCTAAATAGCTTAGACATGAATGCA	P	GAATTTTGGCGCTGATATATAGAGTCT	GAATTTTGGCGCTGATATATAGAGTGTGCAATTTCAATTCGATTTCAATTTAGTCT
58	315	CTTGCACTAAGAGAGCTTTGTGAAG	P		TCTACTGAGAGGGTGTGTTGTT	P	GCTTGATGCTCTTAGTACTTTCAATT	GCTTGATGCTCTTAGTACTTTTCAATTTGTTGGATCTCAGTTTCTTAGACAGAC
59	316	TTGCCACCTGGACACTTTC	P		AGTCGGGTCAATGACCCC	P	CAGCCACACCTGTAAGCTCTTTCTT	CTCTTCTCTCTTAAATCAACAGGAGAAAGAGGTATAGCATTTGGCTG
60	317	AGAAATTCAGGCTGCTGCA	P		CTCTGAGTCAATGACCTTAAGCA	P	TTAACTGGTGTGTTTGTAGATTATA	TTAACTGGTGTGTTTGTAGATTTACTGTGATATATTTGCTTATATGTTCTGTAT
61	318	TCCTTAAAGAAAGCTCCGAG	P		CTCTGGGTACAGAGGCTG	P	ATCATTAAGTGTGTTTATTTTGGGC	ATCATTAAGTGTGTTTATTTGGCCGCTAGGTGAGCAGAGCCCTCTATACTG
62	319	CTTGATGAGGAGATCTGGGTTAC	P		CAGTACAGCATAAAACTGTTCTGG	P	CAGGTCCACCAAGAGCTCTCATCT	CTGAGTTCACTCCAGACATTAACAGAGGAAATAGACATCTCTGTGGTGGACCTG
63	320	TTATTTCTACAAAAGACATATGCACTTG	P		CATTAATACTGTGTGATGTACACATTT	P	GAGTCCAGCTGATGCCCATCAACA	CAGTCCAGCTGATGCCCATCAACATGTGATATTTGGATCAATAAAAAATTTGTTGA
64	321	AGAACTACTGGCATCTTTTGGG	P		GCAAGAGCGAAGCAACAAAGC	P	TGATGAAAGAGAGTTGCACTTGGGG	TGATGAAAGAGAGTTGCACTTTGGGGGCAACCTGCTGGGCTCCCTTCCGATT
65	322	CCGGGATGTCAAAAACCT	P		GGAAAGAGATGCAATTTTGGG	P	TTTTTAAAGTTTGTAGAGCACTTTGT	CGAGTTTAGAGCTCTTAATGAATCAAGAAAGCTCTCAACAACTTAAAAAC
66	323	GTGTTCCAGAGACTTTATGACATC	P		AGTTTGTGTTCTGATTCTATGTCG	P	ACTGGGCAATTAATTAAGTAACTTC	TCAATTATGCTGCCACTAGAACTTAAAGAGGTTAAGTATTTATTTAGTGGCAGCT
67	324	AAATCTTATTTCTGCCCTCATG	P		CTTATGCCCTAGTTTCTCTGCTAGTTTCA	P	ACTATTAGTCAATTAAGTACTTCATTT	ACTATTAGTCAATTAAGTACTTCATTTCTGCCCTAGGTGGGAAATGAATTTAT
68	325	TTGCTTTTACNAAGATGAGC	P		ATTCTGATGCTGCTTCAAGTTGA	P	GGAGCATAGGTTTGTCCAGCAGG	GGAGCATAGGTTTCCAGAGCAGGCCCTCGGGCCAGCAGACAGACAGAGAGGT
69	326	GTCCCTGTAGGATACACCAACC	P		AGACCTGGTTTCACTACTGATGATAGC	P	TAATTTAAAGAAAGAGACTCAAAAC	AAATCAACAGGGAATAATTAACACTTAAGTTGAGTCTCTTTCTTTTAAATTA
70	327	CATATCCCTCAAGTCAAGACTCAGG	P		CAAAAAGCTCAAGCAAAATTTGG	P	CTCAAGCAATTCGAATCGGCAATTTTC	CAAGCAAAATTCGAATCGGCAATTTCCGTTTGGCTCAGATCAATCTCAGAAATC
71	328	CTGAGATGTTTCAAAAGTACGA	P		AGAGCGAAGAGGAAACACACAG	P	CAGAAATTTTAGAGAGCTAGGAAA	AAAGCGAGTCAATCAATGAGCTGAATTTTCTGACTCTCTCAATATTTCTG
72	329	TGCTCTTCAACTCTGGGC	P		CAATTAAGAAAATCAAGCAACCTTG	P	CAAGTGATCTCTCTGCTCAAGCTC	CAAGTGATCTCTCTGCTCCCAAGTACTGGAAATTTGGACATAG
73	330	CTGTCTTTTTCTAATGTTTCTTC	P		ATTTCGGTTTGTATATCTCTCA	P	ATAACTCATATCAGATCAACACAAA	ATAACTCATATCAGATCAACAGCAGATAGAACTGATGTTTCTGAG
74	331	ATCTAATCTTACCAATGTTCTTCATGG	P		CTGTGATGTTTGTCAACAACCTTTC	P	CGCTGCCCTGTAGGAGCTTACCCTAC	CGCTCATCAAGCTCTGATTTGATAGGCTCAGGTAAAGTCTCAACAGGCGAGGC
75	332	TGGAGTAAGTAGGGGTTCCACA	P		AAATAAATTTTGGCATAGCCAACTTGG	P	AAAGCTGTTTCCACCATTTGATGATT	CAAGGCATCTTTACCCCTTGATGATAGATCAATGGTGGGAAAACACCTTT
76	333	CAAGTTCACAGGCCCA	P		GGCAATTTGATGGCGAGTGG	P	XCCTAGCACCCACAGAGCAGCAGCA	CCCTAGCACCCACAGAGCAGCAGCTCCCTCCCTCCCAAGAAACAGCCTCT
77	334	TTGCACTGAGTAAACAGGCCA	P		CAACTATTTAACAGTGCACMAAA	P	TTGCAAGCTTCCAAACTTTTCCCGAGCT	TTGCAAGCTTGGGAAGAGACTCAAAAGTCCGCGCAAGTTTTGGAAAGTGA
78	335	ATCTCTGCACTCTCATGTTCAATTT	P		GTGTGTGTGTGTGTGGCC	P	CTGCAATATGATTTGTAGTTGTTT	AGCAATTAAGTATAGCCAAATATGAAAACAACTAAACATTAAGTACAG
79	336	GTAATATCATATAGGACCCCAACTAAGT	P		GTGTGATGCTGAGTTTGAATTTTC	P	CTGCAAGACTGATGTTGATATCTGCG	GTCAAGAACTGATGTTGATATCTTGCCTTCAGACAGATAAACTAAATTTGCAAA
80	337	ACTCCGAGTGGGTTGTGMA	P		TAAGGCTTAATATCAACAGAACAC	P	TTGATGTTCCAGTGTGGTGCAATAGMAATTTAGGTATTTTAGGCTAGTAAA	TTGATGTTCCAGTGTGGTGCAATAGMAATTTAGGTATTTTAGGCTAGTAAA
81	338	CGGGGATGCACTATCTTGT	P		CATAACAATGCACTAGATTAATATCTCC	P	TACAGTCAATCACTGAAATTAATATG	ATACATATTTAGGAGGCTTTTAAACACCAATATTTTCACTCAATGCACTGTA
82	339	ATCTAGAAATAGCAAGGCTGAATCTGTAA	P		GAATGGGAGTACAGCCTACCC	P	ACTGGATCTCATCTTCTTCAAGAGCCT	CTGAGTTGACAGAGCTGCTATAGAGAGGCTGTGTAGAGAAATAGAGATCCCA
83	340	AGTGAAGTGAAGTGCCTGTACAG	P		CAATGGCAGATATGGTGTATGCG	P	ACCTTGACTGCGAGTTCAATGAGGCC	AACACCAAGAAATCTGCACTCTCGCCCTCAATCAATCAACCTCGAGCTCAAGT
84	341	CGGGATCTTCTTTAAACCAAT	P		GTAAATGATTAGATGTACTATTAAAGGTAAGT	P	CTCGAAATTAAGCGAAGAACTAACA	TATCTATGAATTTTGGAGACAAAAGCTGTTAGTTTCTTCTGCTTATTTTCGAG
85	342	CAACATGGTCAAAACCCCATC	P		CACCTCCCAAGTTCAAGAGAT	P	AAAAATTAGCTTGGCGCTAGTGGCTGGG	AAAAATTAGCTTGGCGCTAGTGGCGCGCTGTAAATCCCGAGTACTCAGGAG
86	343	TCACTAGACAGTCAAGCTAAATAA	P		TGGTGTGAGTATTAAATTTGTTATTGGAG	P	AAATTAAGTCTGGCGCTAGTGGCTGGG	AAATTAAGTCTGGCGCTAGTGGCGCGCTGTAAATCCCGAGTACTCAGGAG
87	344	TCCCATGCTCTGTCTTCTCC	P		GGGTTGGGAGCTCCAGCA	P	ACTTAAAAAAGAGAGAAACAAGAA	ACTTAAAAAAGAGAGAAACAAGAGCAGCTGTCCAGCAGAGCTCTCAATACAA
88	345	TTATATAAATTTCTGGATACAGGCTCC	P		CCCTACACTTTTCTCTCATAGCCA	P	CGCGCCTTGAANAATGGAGTTTAC	CGCGCCTTGAANAATGGAGTTTACGNTTTTCAACCGGAAAGAGGCTGGCT
89	346	CTGAATAGAGATTTGGGAAAAAGAA	P		TTATATAACTCTTCTCATTTTCTGGCTTT	P	GGCAATTTGGGGGTGGTCTGGAGAGGCTACATTTTAAACCAAGAACCAAGAAA	GGCAATTTGGGGGTGGTCTGGAGAGGCTACATTTTAAACCAAGAACCAAGAAA
90	347	GCCTCGGCAAGAAAGACAC	P		TTTTTCCGCAATCTCTATTGCTTAC	P	TGAGTTACCCCTTCTTATTTCTTCA	GTAATTTTAGTTTGTGCTGTAAGTACGTGAGCAAAATTAAGAAAGGGTAACTGA
91	348	AAAGCTCTTCTTGGACATGAGCA	P		CATTTAATCTGATCAAGCAATCCC	P	AGCCTGTGCCAGCTCTXAAACTTGGCTTA	AGCCNCAATTAATCTGCCACACTCTCTAGCGAGAAATTTAAGCAGCTGGCAGACG
92	349	GAGGACACATCTGTCTATTGGATG	P		TGTACTTAGCATACAAATCTCTAGGATGATT	P	ACAGGCCAATATATGTTACTAGAGT	ACAAGCCAAATATGTTACTAGAGTAATTTAATCTATTCTATTCTCAATCATCC
93	350	CCRTATTTCTTACAGGAAATAGTGAATTTTA	P		AGCTCCCAATACCTGTTTTC	P	AGCTCCCAATACCTGTTTTC	TAAAGGCTGATTTTTTTTCAAGAGTGGCTTATAGACATCTCTATATADAGCT
94	351	GAAACTATAGACACAACACACACAT	P		TTTTGATGTGAACATTTTAGTGTATAA	P	GGCTATAAATTTCTCTCTCAACACTG	CGAGNATCTGGGACACAGCTCAAGCCAGCTGTGTAGGAGAGCAAAATTTATAGC
95	352	GTGAAGATCGAATACCTAAATATCTTATTAC	P		AGGTTTGTGTTCCAACTCCMAGG	P	TGAGATTAACCTCTTCACTGACTGCT	ACATAGGTTTAAGTCTATGCTTTCAAGGAAAGTCAAGTGAAGAGGTTTAATCTCCA
96	353	CGCGGATCTCTACAGAGAA	P		GTATTTTGAATGGAAATTTGCATTG	P	TKTTTGGCTTTTGTGTGTTTCTCATC	ACAAACCACTGCTGGAAGAAATCAACCGATGATGAACAACAACAAAAGCAAGA
97	354	GCTGAGAGAAATACATGATGAAC	P		TGTATGCAATGCTGTCTGGGC	P	CAATATGGTCTTTTCCACATGTTT	AAACATCCATGCTCTATGAAATGGAGMATCATTTGTGAAAAGGACNCAATTTG
98	355	TTGCTCTCCATCCCAATTTCTGGG	P		CAITTCGCACTGCTGAGCTCC	P	CGAAATATTTTCAAGTCAATGTTT	CGAAATATTTTCAAGTCAATGCTGATTTTCAAGCAATCATTTTAAAGGACACAG

Row#	REF#	Upper PCR primer	1	2	Lower PCR primer	Modified	3	Modified gBA primer	Flanking sequence
99	356	TAAGCGAGGAGATCACTTGA			TTTTTTTTTGAGATGAGTCTTGC		P	ACCAGAGGTGGAGGTGXKAGTQAGC	CCAGGAGGTGGAGGTTCAGATGAGCAGAAATTACAACAACCTCAATTCGAGGC
100	357	AAATGCCGCACTGCTGAG			CCTCATTTGTGAAATCGCAAGTAA		P	CAGAACCCNATGCATAGTGTATTTC	ATAGCAATCTCTCAATTAGTCTCGGATACACTATGCATTTGGTTCCTG
101	358	TTAGCGGAGGATAGACGATAT			GCTATAGACAGGTCAAAATWACTAGGCC		P	GCTTAATATAGCTCAGAGAGGACGAG	CCCTATTTTGTCTGCTGAGCGGCGCTGCTCCCTCTGGCTAGAGATTAGCG
102	102	TTGGCTTACTGCAATCTGACTCT			GAATCAACATGAGAGAAACCC		P	CCATCTCCACCTAAAGAAAGAAAAA	ACCACATAGCTCGCTTAACCTTTTTCTTTTTTTTTTTAGTGCAGATAGG
103	360	TTAAATGTTATTGCGTCTCTACCC			AAATGTAGACATGCAATCTACTTAGGAA		P	ACTTAGGAAATATTATTAGAAACATGA	CCCTATCAGAACTCCCAAGCATGTAGAGTCAATGTTCATAAATATTTCCTAAGT
104	361	TAATATCTCATGAATAGAAATGGCTA			CAATTAGCTCTCAGAGCTCAGCGCT		P	CCAGATAGCTCCCTCATATGAAAGT	CTCATACCAAGAAATGCAATCAAGAGCTTTTCATAGAGAGGTCAAGTATCTGG
105	363	GAATTAATTTCTTGCATCTGTAGACA			CATAGCTCATTTGGGGAAGTCAATTG		P	TTGTTXGACATTTAAAAATCCGCAAAA	ATCAATTCGCTTAACATTTGTGTAGTACTTTTCTGCTGATTTTTTAACTCTCCAAAC
106	364	TGCAAGAGAGATTTTGAATCCC			GACTGGCTAAITTTATGAAGAAAGAGTTT		P	CAGGAAGCATTTACTGGGAGCATCA	TCACATTTGGCCCATCATTTGTGTGCTGATGCTCCGAGTCCGAGTAAATCTGCTGC
107	365	ACCACCATGTCTGGTTAGTTTATTAA			CATAAATTCAGGCACTCAGGAG		P	GCGTCAGGCGAGGATAGCTTTGAT	TCCGAGGTAGTCTCAAAATCTTGGGATCAGACTATCCTCTGCTCTCAGCC
108	366	TAAGTAAATCTGATTTTCTTTTAA			GTCTTGTCTCAAAATTAACCCAAA		P	CTCAAAATCTCCACTCCGCAAGGATGG	CTGGCGGAGTTAGCGTCAACCCACCCCATCTCTGGCAGGTGGAGGTTTG
109	367	GTAGCGATCAATTTTCTTCAAGCTG			CTTTATTCTCTCCCTAGCCTCATTG		P	CTAAATGAGGGCCATTTCTCCTTGA	AGGAGAGAGAGGGGTCTCATTTTGTTCGAGGAGATTTCCGAGTCTCATCATTT
110	368	AAATGCCCTGCTAGAGGGAG			CTAATCTATCTCTCAGCTGCA		P	GCGCTGTGGGAATGTAGACTTTTG	GGCCTGTGGGAATGTAGACTTTCTTGGGGAAGATTTCCGAGTCTGAGTAAAGAA
111	369	TCCTCATATACATTTTAAATGCGACTG			TGCCCTGCTCAGCTGCA		P	AGTTACAAAATGAAAGACACTAAAT	AGTTACAAAATGAAAGACATAAATCTGTAGTAGAGTTTCAGAAATCTGTGGC
112	370	TTGTGAGGCAACCCGATATAC			CTATACAGCTGTACTCTACTCACAACCA		P	AGTCCCAACCCGCAACACACACAACA	AGTTTCTTGAAGTCTTGAAGTGTGAGTGTGTGTGTGTGTCGGGTGGGAGCT
113	371	TATGTGAGGTCAAGACAGTCTGG			CCAGATATGACGTGCTCCCTC		P	TCCCTGCTCAACACATGTTATATGGCT	TCCATTTAAACCCAAAGGCAATGGGAGGCAATAACGAGCTGTTGTGAGCAGGG
114	372	CAGTTGTGGMAACACTATTAATTATGACTAT			GCAGAGGATCTAGACAGATGATTTTG		P	COMATGAATTAGCGCAACTATCC	COMATGAATTAGCGCAACTATCCCTCTAATCTCAAAATTTTTTGTGTATCC
115	373	CGGGGATCTGMAAAAAAATTC			AGAGCGAGATTTGTCTCACTTG		P	ACTTTATAATGATTTTCACTGCACTC	ACTTTATAATGATTTCACTGCACTCCCCCAAAATTCGAATCCCTCAATTTTACA
116	374	TCCAGATAGTTCCAGAGACTTCA			CTTTAGCTTCAGTTCCAGATGACTGTT		P	CTTTTACTATGTTTTTGTCTCTCC	ATGAGCAKATGTAGTGTATATGGGAGTGTATGGGAGGTCACTCATTTTACCTATGT
117	375	TACAGAAAGATATCTGAGAGGCT			CAAAACCCCTTAAGCCCACTGA		P	ACTTTTGGAGAGTTTTGGGCTGTGG	ACTTTTGGAGAGTTTTGGGCTGTGGAGGTCATCATCATTTTACCTATGT
118	376	AGACTGCTGTGATGGCTTTTGACC			CTAGGCAAAAATGCACTAGTTT		P	GATGAGGAACCTTGTGGGAATGGA	GATGAGGAACCTTGTGGGAACTGAGTAAAGTCACTCTTCTGTAGGCAAG
119	377	TATCAGTCTCTTGAAGCTATGGG			CCGAGAGGAGCACTTCAA		P	AGAGCTCCNAATCCAGCCCTCAATTCAC	GGTTCCAGGCTGAGCTTTCTCAATCCGTGGAATGCAACTGGAATTTGGAGCT
120	378	CTCCCTGTGGCGCTTCTG			ATCTTGGCATGTAGACACAGAGAA		P	GAAAGAACCGAGGCAAGGTGTGTA	AAATGCGAGAGGCAACCTCAACCCAGGTACAACACTTGGCTCTGGTCTTCTTC
121	379	CAGATCAACAGACTAGAAGGGAGTT			ATCGAATTCAGAAAGTAAATCGTGTG		P	TGGTGCATGTACTGCTGTGTGTG	TAAGTAAAGTTAAACAACAACAACAACAACAACAACAACAACAACAACAACA
122	381	TTTGTGTTTGAAGCTTTTATAGCTG			GCAAGCTCAGGGGAAAA		P	GCTAAGAGTTGGCTCAGACACACC	GCTAAGAGTTGGCTCAGACACACCAGTGTAGATGCTGTTTCCCAACAACA
123	382	AGATGATGTGAGTAAAGGAGTCT			CTCTGTACTTATTATCTCTTACCTGGGA		P	TAATCTCTCGCCCTATCTCTTTTC	GAAGTTGTGTAGAAAATACTACAGACAGAAAGAAATAGGCGAGAGAGATTA
124	383	AGACAGGAGATGTTTCTCTTATTCTC			GTGAGTACTCTCAAGATGAAAAATTT		P	GAAATTACAGACTCTTGTGTXANTCCAG	GAAATTGTGCTTAAATATGCTCATCTCGATGCAATGCAAGCAAGTCTGCTAAT
125	384	CATCGAGACACCGGGTGG			CCAGAGATCTTTCAATTTCTGTGTTG		P	AAATGACTACTGTATCGCGCTTAC	ATGGCTCGCGGATACATCTTGAACTGAACTGGAATGAGTACTCAT
126	385	GGGATCTCAAAAAAANAAAAA			GATCTCAGCTCTCAAAATCTCTGTC		P	AGTGAATCTCTCAGCTCAGCTTCC	GATGTAACCTTCCAGCTACTCGGAGAGCTGAGTGGAGTGAAGAACTACT
127	386	TAATTTCCCAATTAATCTAAGTCCA			CNAAAGNAGAGTGCAGGG		P	CCCTTTAGACCCCACTCTCTGCTCT	CTTCTGTGCAATTCAGAGAGTTAGAGACAGCAAGAGTGGCTTGTAAAGCG
128	387	CGGGATCAGCCCAATTCMA			TCAAAGTGTCTCATTCACATGTC		P	ATTGAAATATTAATGTGAGGGC	ATTGAAATAATTAATGTGAGGGCCAAAAGAGTGAATCTGTGTACTGCA
129	388	ATAGCTGAAAGATATGAGACTCTGGG			CTTAAGCACTGCTCAAGAAATCATGA		P	AAATCATGCACTCTGGCTATAGTC	GGAATCCAGGCTTACTCAGACGCAAGGCACTATAGCCAGTATAGCCAGTGTGATTT
130	389	ACAGGTGGGAGTGTCTGT			AACAGACCTGCTCAAGAAATGAGCC		P	TTTTGTAGAGAAAGGCTCTGCTCAT	TTTTGTAGAGAAAGGCTCTGCTCATATGTCGCCCAAGGCTGTGTTTCCAAAGTCT
131	390	TTGCTGATTAATTTTAAAGCATCTTTG			AGACATAGGACACAGACTGAATTTCC		P	CTTGTAGAAAACCTATGCAAGCTAGAG	AGTATACCACTAAGAACTTGGCTAGACTTCACTGATTCATAGTTTATTACTGA
132	391	TATGCTATTAATTTTCCAGGGGATTC			ATTCAAGAGTGAAGCAACTTTCAACC		P	GGTGTCTCATGTTATGAGTTGGCACT	GGCAATCTTATGTTTTTATTTTGTCTAGACTTGGCACTGCGCACTTAAGTGAACA
133	392	AGTAGCTAAGACTACAGCACTGTGC			CTAATACCAAGTCTTTGGGAGG		P	GCTGGGAGTTGGGTGTTCTAGTTTA	GTTTTTATTTGCTABACTGGGCAACCTTAATAGGAAACCCCAACTCCCGAGC
134	393	GTGACTCTAGTGTGTACAAAGTGA			TACCTGATCTAGAGGCTTGTGTAAGAAC		P	TCTCTAATCTTCCAGAGGCAATATG	TCTCTAATCTTCCAGAGGCTTCCAGGCTCTCCAGGCTTCCAGGCTTCCAGGAGAGG
135	394	CCCAAGTGTTTACCCAAAACATACTAA			TCATGAGTCTGGGAGTTG		P	CTTCTTGGCTTTGAGTCTGCA	CTTCTTGGCTTTGAGTGTGAGAGTGTGGAAGGCGGCGCAAGAGTATAT
136	395	AAATGCTCAAAATTAAGTGGGCACTCT			CTTCTTGGCTTTGAGTCTGCA		P	ATATACTTTCTGCGCCCTCCACTA	CCACAACAAGAAAGAGAGCTGAGATTTGGGCTGAGATTTTGTAGACATGAAGAGTAT
137	396	ATATGGGATCTTAGTGAGTTTGTCTT			ATTTTCTTCTCAGCACTGATGC		P	AAACCTCTCATATTAATAATCTAGCC	CACAACAAGAAAGAGAGCTGAGATTTGGGCTGAGATTTTGTAGACATGAAGAGTAT
138	397	TTCCGAGAGCTAGGGGCTC			CGGTAACCGGGGATCTGATG		P	AAAGAGATATCTATCTGTGAGNAGCA	AAAGAGATATCTATCTGTGAGAGTGTGGAAGGCGGCGCAAGAGTATAT
139	398	CTGTGTCAAGCAACATTTGAGG			CATTTAAGAACCACTGAGTTTACTGACA		P	TCTTGGATTCGATCTGTGAGNAGCA	AAATGAGTATCTATCTGTGAGAGTGTGGAAGGCGGCTTCTCTCAGAGTACGATCTTCT
140	399	TGGGTACACACAGGGGATCT			CAITTTTGTGATACAGATTAAT		P	CAAGCGATGATTTXCTCGGAAATTC	STAGTAATTGACTCTGAAAACCCAGGCGTAAATTCACAGGAAACATCTACTGGCT
141	400	AGCTTACATTTCTGATCATGTTCAAT			CAATTTTGTGATACATTAATCTTACCAAGCC		P	AGTCCACTGTGCTCGGCTGTCTACT	AAATGGAGTGTCTTTAGAAAGTTTACATGAGTAGACACAGCCGAGCAAGTGGAGCT
142	401	ATGAGAACCTCTGGGATATAGCA			CCTTTTGTCTGTGCTGCTCCTAACT		P	AAGCATTTGATATXATCAAGATTAATACTG	TCTGTTTGCATTTGCAAGTGGGCTCCGAGTATTAAATCTTGTTAATTAATCAAAATGC
143	402	ATTATAAGTACAGAGGCTAGAGGGAGACA			GACATAAAAAATTTGGATAGAAGCAATTTG		P	ACCCCTCACAGTCTCATCTTTACT	TCCGAAAAGAGTAAAGATATGTATAATGGAAATATAGATATGAGACTGTGTGGGGT
144	404	CTTGATAGGCGGCTTTTACCTT			TTATATCATATGGGAGTACACCC		P	CAAAATGGCTXCTCTTACGGGCTTTATG	ACCCTGTGGACTGTGAGCTTACGGGCTGAATAAGCTCCGAGTAAAGCTCCGAGAGAGCAAT
145	405	GTGATACAGGTTTCAATTCCTAAGCA			CTTTGTGAGGAGGAGTAACTTCAACA		P	CCTCTGAGAAATGGGAGATATGATG	CCTCTGAGAAATGGGAGATATGATGCTCTAATTAAGCTGTGAGATATATCAAAATAT
146	406	ATAGTGCACAGCTTTTATCCCTTTT			CGGGGAGGAGGATGCTCAATTTAA		P		
147	408	GAACTGCAAAATCTCAATATCTTATTCTCTG					P		

Row#	REF#	Upper PCR primer	1	2	Lower PCR primer	3	Modified	Flanking sequence
148	409	CCGCTGAAATTTATATAGTACTTTTTTTTGG	P		TCACCCAGTTGCGAGCTTC		CGGCGCCTTTGTTTACCAACTGTTA	AAACTTCTATTATATACATATATATCTAAACAGTTGTGTAAACAAAGCGCGCG
149	410	GGAGCTTCGAGTGAGCTGA	P		CATCAGCAACTGTTTTCTGTTCTTTTT		TTTTTTGAXAGCGXGTCTCCCTCTGTTC	ATGSCGCACTCCAGCTCCAGCGTGGCGACAGAGAGAGCTCCGCTCTCAAAA
150	411	AGAGATTAGTTGTGGCGCTTGG	P		GCTTCGAGCACTGCTGCTCGAG		CCACAAATTCGCAAAATACAGCAACA	GTTGCGAGGATATATCTCTTGTGTTGGGTGGTTCTGTATTTGGATATTTGTGG
151	412	TCAGAGCAAAATAAAAAAGGCG	P		AAGCATATGCTGCTGCTCGAG	P	TCACACTCTACCCAAACAGCAAAA	TCACACTCTACCCAAACAGCAAAAACCTTTCTTCTCAAGATATCTGCGAA
152	413	TGTGATGTACGCATTTTATTATC	P		GTATATGACTGAAAGAGTCAGTTATTCTACG	P	GGGCGCTAGGCTGTACTCCCTGCTGTTT	GGACCTTAGGCTGACTCCGATGCTCTTGTCTATTGTATAGTAGTGTGCGATGAGAC
153	414	TTTTGCACATTAGCTCTTAGG	P		GATTAGCAACACACATGTTTACAGCA	P	AAGCGCTTAAATCCAGAGAXATGTTTAC	GCGCTTAAATCCAGAGATATGTTTACGCTCTGGCGCTCCGCTCTCTTAAATA
154	415	ATAGACCTAAGCGCTTAATCCAGAG	P		GTAAACATTTCTCTGCTTACAGACAGGT	P	GGACTCTACGACAGGACATTTATA	TTTTTACTTCTCGCCCTCCCTTCTTAAATTAATCTCTCTCTGCGATAGTCTCC
155	417	ATATATTTCCAGGAGCTGCATCTT	P		CTTATCATATTTTTCTCTGTACCATTTCTTTAC	P	GTTAAGCGGAGCTACTGCTGTATACCA	GGTAAAGGAGCTACTGCTCATACAGACAGTACGAGAGTACGAGTACGAGAG
156	418	TCCTCGAGGACACAGAGNTT	P		CATATAGGAGTTTATCTAAGGGAATACCA	P	TATGCGCTACAGCTGCTATATACGCT	GTATTTGCGATGCGAAGAGTGGAGGGTTATAGGAGTTGTAGGGGATTA
157	419	ATCATAGGCTGTGTTGGCA	P		CAGAGCCATAGAGTTCTCGG	P	GGGAGACAGAGGAGAGACATGGCG	GATCTTCCCGCAGGCTCTGTGTCGACGCACTGTCTTCCCTCTGCTCTCCCG
158	420	AGAGACAGAGGCTCCTTTCCAAA	P		GTATTTTTTGTAGAGTCGAGTTTGGC	P	AGGTCGTGATGGGCTAGABAGCAA	AGGTCGTGATGGGCTAGABAGAGTCTCAGCGCTGGCGACACAATGGCAAA
159	421	GAAGGCTGAAATGATATTCATTTGT	P		AGAGACAGCTGGGAGCAT	P	GTGAGTCTCTTCTTTTAXTTTTTTTGA	TTTTATGCAAAACAGTATGGACGTTCTCAAAAATATAMAGCAAAAGCTAC
160	422	CAGCCCAAACTCTACAGCA	P		ATTTCGATGACACCTTACGCTTACA	P	TTGCAATCATTTATTTTATTTTTATAG	CAATCATTTATTTTATTTTTTATAGCTCACAGCTGGCGTGTCTGTCTTTGT
161	423	TGCCCTCTCTCTGTACAGC	P		CTGACCTCTTTTGTAGTATGAGGCA	P	CAGGSAACAAGCCAGRCCTGTGTAGCA	GTCGAGAACTCTTGTGCAATTAACAGGCTCACAGGCTCACAGGCTGTCTGTCTTGT
162	424	TCACATATAGCAGATCTCGAGC	P		AGATGATCTTTTGGGTGAGCAGCA	P	GGCACAAATCAAXAACAXATTTATTA	GTCTTCTTAAATATACATATTTGTCACTGCAATAATATGTGTTATGATATTTGT
163	425	GTATGTGATGCTGCTGTAAAGTTAGTGCT	P		ACTTCAAAATTTACATAGCACACATATG	P	TTAAAATTAAXAAAATTTAGATTAAAG	AAAAATAAAAAATTTAGATTAAAGCTAAATTTAGAAAGAGTTACTTTTTGAA
164	426	TAGTTCAAGACAGGCTGACG	P		GTTTCAAGCAATTTCTCTGCG	P	CCCAAAATAGCCGCGTGGCGCATCTCCGTAAATCCGACATTTATGGG	ACAAAATAGCCGCGTGGCGCATCTCCGTAAATCCGACATTTATGGG
165	427	GCAAGTATCCGACATGTAAACA	P		TCCTGCTATTTCTGTGCTTAATTTGTT	P	TGATTAATTTAAACAATTAACAGAG	TTTTCAAGCTACTTAAGAANAACAGACTCTTGTTATTTGTGTTTAAATATATCA
166	428	TATAAATGAAATATTTTATGATCCAGG	P		TTTACATTAGTTGCTTTTCTCTAGGTTG	P	CTGAGTGTACACATCATTTCTCTCC	CTGCTCACTACATATATCAGCAACGAGGAGATGATGTTGTGTCACACTGAG
167	429	TGTTCTAGTTAGAGCAATTTACACTAGGAAAA	P		CCTCAGGTCACACATCNTC	P	AATGGTAAGATAGTGTAAATCAAT	AATGGTAAGATAGTGTAAATCAATTTTTCAGTCCAGGCGAGCGCTGCT
168	430	CTCTGATTTTACATGACGATTTTGTCT	P		TCCTAGRCCTCCCGAC	P	ATACAGCTTTAAAGAGAAATCGTTGA	ACAGATTTAAAGAGAAATCGTTTGAGAAATACAAATTTCTCTTTTTTTTAAATA
169	431	CAAAACACTCTATACGTAATTCCTTTT	P		TKACCTTGATTTATACATGCTAGGATC	P	TCTCCATCAAAAGATTTACTGTTAAAG	TCGACAAAAGATTAACGTTTAAAGCGCCTTTCTGCAATCTGATGATGCTAG
170	432	GGGATCTCTGGGAGCGCT	P		AAATTGTGGCTTGAAGGCTAAATTTCTAT	P	ATTCTTTTAAAYTCTTTGTGAAA	AGCCACTGTGAAAGCTGTTTGGAGTTTTCACAAGATTTTAAATAAGATAT
171	433	TATTTATAGGCCATCTCTGCAATTCG	P		CACCATGCGAGATGCGGTC	P	AGGSAAGACGATTTAXTTTGGCTCACAG	GAAGAAGAGATTTAATTTGGCTCACAGCTCTGCGAGCTGTGCAATAGTTAGTTCTAG
172	434	GTACCGGAGCTTTCTGCTTGG	P		TCTTTTGGAAATTTCCAAACAGCT	P	GGCTTAACTAATTTACATTTCCACCAA	GAAGAAGAGGAACTAATTAATCATAGTTAGTTGGTTGTGATTTAAATAGTTCTAG
173	435	TGGAGGTTTGTGTCGCAAC	P		TAAATGCGCTGGAGAGCTCTC	P	CTCAGCAGTAGAATCAAAACAGTAG	TCTTGGAGGCTCGAAATCTTTGTCTACTATTTGTGATTTCTACTGCTGAG
174	436	GAACCTGCCCAATGAGC	P		GTGTTCCGACCAATCTCA	P	CAATACCTCCCAACAGCTCCCTCCCT	ATACCTCCCAACAGGCTCCCTCCCTGGGCAATCGGGATATACATTTCCAGG
175	437	GCTGTCATTTGCTAGTAAATTTGA	P		TTAGCTTTCTGAAACTCATATGTTGA	P	ATTATTTGAATATGCGAATTTGGGA	GAGCCTGTATCCCTTTACAGTAGAAGTCCCAATTCGCAATTTCAACATAAT
176	438	TATATACAGTGGGCTCTCTCTTG	P		CTTTTAGCAGTATGAAAATTTGGAATATACACT	P	CCCTTTAACTACTGAAAGCTCTGAGC	CTTTTAACTACTGAAAGCTCTGAGCTGCAACGAGAGATTTCTGATTTGCTTT
177	439	CATGTGATACGATTATGCTGATGAAATG	P		TTTTTAGCCTTTTTCGATTTATCCAGTG	P	TATCCAGTGAATGGGGAAGAGGCA	AAATATCTCCCAAAATTTCTTGCACTCTTTTTCGCAATTTCCCAATTTCACTGGATA
178	440	TGATGAAAAGGCAATGTGA	P		GGCTCAAGTTTCAGGGC	P	TCCTTGCATGCGCTGTGGGAGAGA	ATATGGCAATGCACTCTGCAAGTCTCTCCGACAGCCCAATGCGAAGA
179	441	CTAGACATTTTCTAGACACCTC	P		TGAGCGCAAGTGCTCAGA	P	AAATATGAAATTTTATTTGACATCTCC	AAAAAGATTTCCAAAATTTAAAAATGGGAGATGTTCAATTAATAATCTATAT
180	443	CAACCATTTTCTGATATCTGTGA	P		AGAGTGTACGCAATTTTGTGCT	P	TTTTTTCATATATCCATAGAAAG	CTTTTATCTTTTCTTAAATATGTGCTTTCTCTAATGGAATTAATGCAAAAA
181	444	TTCTCTTGATCCGTGGAGT	P		TGCACTGTATACCCAGAGCT	P	AAGATGTGCAAGATCAATGTTTTTCA	CAGATATGATGTGTATTAGCAGGCTGAAACATGATCTCTGTCATGACACTTT
182	445	TTTGCTTCCGCTCTGCG	P		CAATTTAGTGTATTAGCCTCTCTCACA	P	GAGTGGGCAATTTACAAAGGAAGA	CTATGGAACTGTGAGTCTATTAATACTCTTCTCTCTTTGTTTAAATTTGGCAGCTC
183	446	AGAAAGCTTTTACATGCTGGAAGAGAAAAA	P		CRAAGACTTAACAAAGGTGGTCTTAAGG	P	GTCTAAGGAGCAACGAGTGATGCG	CTAAGAAAGGAGAGTTTCAATAGAGGGTACACTGGTGTGTTCTCCCTGAGCT
184	447	AGGAAGTCTTTGACTCCCATCTCTC	P		AAACCCGCTATCTTATTATAGCA	P	CTCATCTCAGACTAXTTCTCTCTTAA	CATACTCAGACCTAATTTCTCTTACAAAGGCTCTCTTGGAGAGATATGTAGCT
185	449	CCACCTCTTAGCAATTCGAG	P		CAAAATCAAAACCCCAATTCAGATAC	P	TTACAAATCAACATGAGATTTGGGCG	TTACAAATCAACATGAGATTTGGGCGGGGCAATAATACCCAAACTATATGAA
186	450	CTGGGCGCAACTGCATTT	P		AAGCTGGGTAGTAGAGAAATACATGG	P	CCACCTGGAATTCACACACACACCT	CCACCTGGAATTCACACACACACCTCTTTTATTAATAATAATCCATGTTA
187	451	AAACAGAGCAAAATAGAGGTTG	P		GTCTGTGTCGGTGGCATAG	P	AACCGTTTAGAGTTCCATTTTGTGCG	AACCGTTTAGAGTTCCATTTTGTGCGAGACAGACTCTGTCGCAAAAGAACTT
188	452	CAGAAATGCAATTGCTATTGG	P		TCCCATCTGTCGATAG	P	AAATCTTTTGTATTTCGCAATAGTGA	AAATCTTTTGTATTTCGCAATAGTGAATTTCTGTAGTCTTCTTGTGTTA
189	453	GTCACTACTCTCCACCGG	P		TTTTCAAGTTTCAATGTGCTACTTTTAA	P	TATTTATAGATTAATGATTTTATTTA	ACCATATCAGCTGTATTTATTTATTTGTATTAATAATAATCTTAACTATMAA
190	454	TAGAAACTCGAAAGAGAGGAGC	P		CATACAGSAGAAATTCGAGTTGG	P	GATCGAGTCAAGCAAGCAAGCAAGGG	GATCGAGTCAAGCAAGCAAGCAAGCAAGTAATCCCTTTCTTCTGTTTCTTCCA
191	455	TATGCGTGGAAATGAAAGTATC	P		CTGAAATGGGAGATATGTTGTTTT	P	CAGTCACAAAAGCAAGACCAACTC	TGAAGTTCTCGAAGCAATTTACTTTTACGAGAGTCTGCTGTCGTTGTGACTG
192	456	AAACCCGCCAAGGTAC	P		TTATTCATTTTTTTTCCGACGAC	P	GAGGATCTGCTGCACAGGCAAAAT	GAGGATCTGCTGCACAGGCAAAATCTGTGCAAAAATGCAAAATTTCTGTGCT
193	457	ANGTGGAGATACACTGGGC	P		CTTCTTGTGTTTTTGTACAGACAGTGT	P	GCGTACAGTGAGCTGTGTATAGCACAC	CTGAGTGTAGCTGTGTATAGCAACAGCAAGACTCGAGCTGGGTAGCAGAGTGT
194	458	ATGCTGAAACCCATCTACTATAAAATAC	P		CTAGGTTCAAGCGAATTTCTCTG	P	GGCGTGGTGGTGCATCTCTGTAA	GGCGTGGTGGTGCATCTCTGTAA
195	459	CTGCGTCTCTCTTGGCTTC	P		TTATAAAGACAGAGGTTTAAATTTGGCTC	P	GTAGTGTTTCTGTAGTGGCGCCCTCC	GTAGTGTTTCTGTAGTGGCGCCCTCCGACTGTGATGTTGTTTCTTATAGAGC
196	460	CTAGAGCTGAAAGAGGAGTGGCA	P		CTGCGCTTGGCAGCAAAATTTT	P	TTGAAATGAAATTTCTTTTAGCATAA	TGAAAGTATAAACCCCTTAAAGAGCTTTATCTGCTTAAAAAAAAATTCATTTCAA

Row#	REF#	Upper PCR primer	1	Modified	Lower PCR primer	2	3	Modified GBA primer	Flanking sequence
197	461	AGCTGCTCTTCTGCTGCTG	P	GAAGCTGATAGATTAAGTGTCTTATTAAGAGAGA				AGAGGCTGGCACTTTCTTTCTGTG	----- CACAAGGCTTTCTGAGTTCTCGCAGCAGAAAGAGTCCGAGCCCTCT
198	462	CTGGGGCTTTGTTTGT	P	AGTGACGATATCACCACATACATATAG				AGTTAATGCAATTCCTCCATCAATTA	TTTAAATTTTGTGGAATTAAGCTTGGTAAATTTGATGGGAATTCGATTAAT
199	463	AGCTGATGATCTGCTGCTTT	P	CCAGAGCAGAGCCGCTG				P	CCAGCAGAACTGAGAGAGAGCTGGGGAGCCGAGCTCTCTCGTGAATG
200	464	GTTCAGTTCAATTTATTTCTTTTCCAC	P	TGGTAATCAGAGCTGCTGTTATCCATC				P	AATTGGCTCTAAATTTGATTAATCTCGTTTCCAGGCTTAAATATAGATGG
201	465	ACCTTGTGGTTCAGAGGAG	P	TGGCTCAAGCCGAGCTC				P	TCGAGAGGCTTAAGTAGTGGCTCCGAGGAAGCCCTCGAATGATAG
202	466	TCTGGTTCAGTATGCTC	P	GAACATATGAGATCTCTATCTCTACAAA				P	GCTGGGCTACAGCTGGAACACGCTGGCTGGCTAAATTTTGGGTTTTT
203	468	ATAGAAATGATATAGATCAGAGAAATTC	P	CAACATATGAGATCTCTATCTCTACAAA				P	TTTCTCTCAATTTGTTTCAAAATGTTTCTTCTTATTAACCACTCTC
204	469	CATTTACTTATGATTTTCAAAAGCTCTGCA	P	AGTAAATTTAGACCTAGATAGCTGGAGA				P	ATTCTTCTATCTAGATACACAGCAATCTTAGGTTCTAGAGAGTCAACA
205	470	TTTTTTTGGTATATAGCTAATGG	P	GTACTTTTAAATGAAGCTGTGGACT				P	AAATGCTTACCCAGTCAATGAGCAGCTGATCTTTTCTTTTCTTAAAT
206	471	CAGCTCCAAAAGCAAGTC	P	TCTGTAGCCAGCAGGAGCTGAT				P	GCTGACCCGAGCCCTTTGCTCTCCGCTGGCTCCGAGGAGCAGAGCT
207	472	AAAAAATAGCTGGATGCA	P	GGCACTCTCTGAGCTC				P	ATTAAGAAAAAAGAAAAAGATCAG
208	473	ATTTCTGCTATAGATGTTCTCTTT	P	GAATGTAAGATGCACTATCAGC				P	TTGGACTCCCATGACCTAGATG
209	474	TATGCTGCTGAGTTATAGGCTGAGA	P	TTCTAAATGCTTCACTCCG				P	TAAGAGCCAGCCCTTTGCTCTGCC
210	475	GAGGCTGAGTGTGTTTAAATTC	P	CGAAATCTCCAAAGTAGGCA				P	TAAAGCCAGCACTATCTCTCTGAGCTGATCTTTTCTGCTGCTGCTTTA
211	477	GACAGTTTGGCTGTGATCTTC	P	CGAAATCTCCAAAGTAGGCA				P	TAATTAATTTCTTGGCAGGCAATTTGAAACATTAATTTGCAACTCAATAT
212	478	TTAGTTCAAGTACTGATTAACCTTG	P	GGCTTCCAGCTTACACACAC				P	AAATCAATCTAGTCTGGCAATTTAGCAATGCTCTGAAATTTGTATCTTTG
213	479	TCACAGCAATATGTCATGA	P	GTTCATACACTGATTTCTTTTCCAGTG				P	CTTGAACACTAGTCAATCTAGTACGAGCAATAGACTGAGTGCANA
214	481	TTTTTTTAAAGCAAGCTGCTG	P	ATTGCTTGAACCCGAGG				P	TGCGGAGTTCGAGTGGAGTGGCTGGCTGATCTTACCTCAGCTGCAAGCTCC
215	482	TTTAACTCAGAGGGAAG	P	CTAATATGCCGAGCTGTCT				P	TCGCTCTCTGCTTTTGTGTTTGTGTTT
216	483	CTAACCAAGCCATTAAGAGAAAT	P	TGCAATGAAGTGAATCTTTCTG				P	AAATTTGCAATTTCTTACCTTTTAAGCAGACTTTTCTACCTCAGAGCA
217	484	AAAAATAGCTCTGCTGCAAACTATGATA	P	CAGGCTCTGAGAGCTTTCTG				P	TGSGATTCGAATGCAAGCCAGCCAGCCAGATCAGATTCCTTTCGAGAA
218	486	TATCTTAGTTTGGAGAGCA	P	CATTAATCAGTCTTAATAATTAATGAGTCT				P	GGCTCTCTGCTCTCTCAAAATTCGAAATTCGAGAGGAGGAGG
219	487	AACCAAGTATGGAAGCTTCTG	P	CATCTAAATATGAGAAATAGCAGCC				P	AGATGTCTCAGGAGCTTGGAGAAAGTGAAGCCGCTCTGAGAGTGAAGAGGCTC
220	488	CAACTCAGCTTCCAGCT	P	TGCTTCAACAGCTCAGATTTCT				P	CAAGAAATTCCTGCTCCTCAGGCTCCGAGTCTGGAATCAGGCCCCAT
221	489	GGTTTTCAGAGGCAATCAAGC	P	CTTAATCAGGCTTGTGATATG				P	GTCTTGTGTTTCTGACCAATCTAATAATACCTGCTGCTAATAATTAATTTG
222	490	AGCCTTCAGAGAGAGC	P	CTTAATCAGGCTTGTGATATG				P	TTCTTGTGATAGTCTAGTGTCTAATAGAGTCTCAGAGCTCTGGAAGAC
223	491	TTATATCTAGTATGTAATTTTTCAGGCT	P	AGTGTGATTTCTTCTGCTGATG				P	TCGAGGAAAGCAAGCACTTACCTGAGAACCCCTCGCTGCTGCTGCTGCTG
224	492	CACCTTTATCTCAGAGGCT	P	GTATATCTAGTCTTATAGACCAAGATCCAGC				P	CAAGGCTTCTGGGATTTGAGAGATTTGTTGTTTTCGAGTGTGCTGCT
225	493	AAATATGAGGCTTCTTCTCATG	P	CTTTTCAATTTCTCTCTCTCTGCTA				P	ATTAAACAAATGCTGCTTTGAGAGATTTGTTGTTTTCGAGTGTGCTGCT
226	494	ATAAGATGAGTCCAGATGCTGAT	P	CAAGTGTCTTAAAGGAAAGAG				P	AGACCAAGTGTGCTGCTTACCTATGCTACATTCGCAAGTGTGCTGCTGCT
227	495	ATAGTATCAACTCTCTATGAT	P	CATCATACCTGCTGGAGATG				P	GGGAGGAAATAGCAAGTGTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
228	497	TTGAGTCCAGAGATG	P	GGCAATGGCTTAAAGCAAT				P	AAANAAAAAGCAATTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT
229	498	GACACAAATTTGCCATGCAAT	P	GGCAATGGCTTAAAGCAAT				P	TTACATGCAATTAAGAAATAGCAATGCTTTTCTCTCTCTCTCTCTCTCTCTCT
230	500	TATGACTAAGTTAAAGTCCAGGCTC	P	CATCATCTCTGGGCTCACTA				P	CTGTTTCAAAATGACACATTTCCATGCAAGATGCAATTTGCTGCTGCTGCTGCT
231	501	TTTAAGTTCTGCTATACATGCTG	P	AAACCTAGATGATGGGTTGATG				P	TGCAAGTTTGTATAGATGATACAGTGGCTGCTGCTGCTGCTGCTGCTGCTGCT
232	502	CTAAATACCATTTGAAATGATAAGATGTA	P	AGTACTAGTATGATTTGCTATTTAGTGTAGCTA				P	CTCAACTCACTGCTGCTTATATGTAATAGATGCAATGCTGCTGCTGCTGCTGCT
233	503	TAAATGAGGCTGAGTGGCTTA	P	AGTACTAGTATGATTTGCTATTTAGTGTAGCTA				P	AGCAAGTGTGCAATTTGTTTGTGAGGAGTTTGTCTGCTGCTGCTGCTGCTGCT
234	504	TGAGGAGTTTATGATCAGCAATTA	P	GGGAGTATGAAATTCAGAAATAGG				P	AGGCTGCTTCAAGACTCTTCTGCTTCCGAAATTTCTGCAAGAAATTCGCAAT
235	505	CACTTATCTCCTATGAGG	P	TCTAGATATTTATGCAATCCATCA				P	TTATAGCATATGATTTGGAACATTTGCTGCTGCTGCTGCTGCTGCTGCTGCT
236	506	TCTAGGAGTATGTTATGAGG	P	AGTCTTCTGATGCAAAATGATCCAG				P	CCAGAAACTCATATGATGCTTCAAAATTAATTTCTGCAAGAAATTCGCAAT
237	507	TAGATGCTCTTAAATGCTTTTCC	P	AGGAGAGGAGGAGTTGGGA				P	GTATCTCTACAGATTAATGAGCTTCCGCTTCTCTCTCTCTCTCTCTCTCTCT
238	508	GGGCTTTCCACACAGC	P	CTTCCAGACTTCTCTCTCTCA				P	GAAAGGTAGCCAGCAGTGAAGTTTCAATTCAGCTGCTGCTGCTGCTGCTGCT
239	509	GTAGGCTCCAGATG	P	CTGCTCAGGATTAATACCCAG				P	GGCTGGGCTGATTTCTGACCACTCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
240	510	CCAGCAGCTCAAAACACTTG	P	ACCCCTTTTCTTCTGAGGATGTT				P	ACATAAATTAATATGATGCTTAAATATATTTCTTATTTATTTATTTCTCA
241	511	CCCTGAGCTTATTAATGATG	P	CTTTCTATACAAAGAGAGAGGAGC				P	ACAGATGCAAAATAGCATAGGAAACACACTCTGGAAGAAATTTATCTCTCA
242	512	TGCTCATATATATGAGGAA	P	CATGCTTCTTCTGAGCAACCTCT				P	AGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
243	513	ACATGAAATTTGATGCAATATATGATA	P	TGATGTTAGATTTTCTGGG				P	TCCAGAGAGGCTTTTCAATTTAAATGCTGCTGCTGCTGCTGCTGCTGCTGCT
244	515	TTTATCTCAGTATGCTTCAAG	P	TAAATGACACTGCTGCTG				P	TTCCAGAGAGGCTTCCACAGAGAGGTTTCCAGAGAGGCTTCCAGAGAGG
245	516	TTTATAGGCTTGGTTTATTTGGA	P	GAAGCTGCTGCTGCTGCTG				P	TTTGAAGCTGCTATTAAGCTGTAAACACTTTACACCTTACCTTACAGAGATCA

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21

Row#	REF#	Upper PCR primer	Modified	Lower PCR primer	Modified csa primer	Planking sequence
246	517	TACACAGAGCGCTCTTAAGTATCTGACA		AAAGATGTCTCAAGGCTTCAGAAGG	P	GTAGTACACGACACTCAAGATAGOSCATATAGAGGGCTCAGTCAATCACT
247	518	AAGCAGCAGCAGCCGACCG		GAATGTGTCACCTCCCATAGC	P	TGGGGTCCCTGGGGCAGACCAATGCGCCCTTTTCCCTCGCAGAACTCTGGGCAC
248	519	GCTAATCCAGTGAAGTTAAGTGTAAATT	P	CACACAGCTTACTAAATTTTCTTAATTA	P	ATTAGTAACCTGCTGCTTGCCTCATAGTCTAATGCGGTAGTTTAAGTGT
249	520	AGAAATGCTGTGAACCGG	P	CCAGAAGAAGCTATTTCAGGCTTAGT		CACAGAGTGAAGCTCGGTTTCAAAAAAANAANAAGAAAGAAACAACTAA
250	523	TTAGCGCTGACCCGCTC		CTGTGAGACTGCTCACTTCC	P	ACGAACTCGGATGCACCTCTTGAGAGGAGCTGCTCACTTCACTGAGAGCTTC
251	527	TTAAGCAGCTTCCGCAAG		CATGGCTGCGATTGCGAG	P	CTAGTAAGCAGAACTGAAATAGAAATGAGCTGCGCTGTCTAAGCTGACAGTTC
252	528	ATGAGGAGCACTTGAGCCC		GACAGCGCAGAGCTCTTGTCTC	P	ACCCATCTCTTAACATGTAACAAAGAGAGACCCCATCTAAGCTCTAACAACAA
253	529	CTCCACATTACGAGGATAGTGC	P	GTAANAATGCTCAGCTCAGATGGA	P	AAAGCCAAATTAACACAGTACAGCTCATATCTCACTAAGCTGCTCAACATG
254	530	AAACATCTGAGCTGGCGGT		CTACATCATCTGATGATTAATAAGCCAA		CTGTGTGAGGAAATGGAATACAGCGGGTTCAGTCTCAAGTACTATTATATC
255	531	TCGTGGCATACAGCATG	P	CACATTTGCGCTAANAAGGCTG	P	CCACCATGCCCCGACCGCAAGAGGATTTCTAATCTCTTGGGTAAATCAG
256	533	TCGTGCGATTTTAAACGATTTATTTG		GAGCGAGGAGATCGCTTG	P	TTCTTTTAGGTAGAGTCTTGCCTGCGCCGAGGCTGAAGTGTAGTGGG
257	534	TAGCTGGATTACAGCGC		GATCACTTGAGGCCAGGAATT	P	AAATTTTGATTTTGTAGTACAGAGGGGTTGTGCCATGTTTGGCCAGACTGG
258	535	TGAAGAACAGCAATTTGATAGG		GCCAAAGGCCAAGCGG	P	TCGATTAGGTTTGTGGCAGAAATTC
259	539	AGTTTCCCTTTCTTTTCCCATG	P	GTAATAATGGCATAAGGACAGGA	P	GACAGGGAATAAAACAAATATCTCA
260	543	CACACACACCCCATACATAAANA	P	ACGTTTAGATATTTTCTTTATTAAGAGATG		TTCTTTTTTAGGCTGTGTTTTATTC
261	544	CTCATCTCATTTCCAGATAGCTNTTAC		TCCCAAGTGTGCGGATTC	P	TTGGAATACTTTTAGATATACAGAA
262	545	GTATTAGCCAAAGATTTGAAGCCA	P	CAGCTCAGATTGAAATTTGTATGCA		TAGAANAATCTGCTTAGTTTAGGCA
263	546	GTATCATAAACAGCTCGCTGCA	P	AGTATGTTCACTTGATTTACAGCTAAGTATTAG		CTACCAAGTATTAGACCTTTTGTAC
264	547	CTTTAAAGTTTCTGTTCAAGTAAATTAT		CTTCAATTAAAAAAAAGTAGAGGAGCTG	P	ATTAGTTTGTTAATCGGTGTTTTGG
265	548	GAGAAATAGGCTCTCATATGTTAGGTAG	P	ATGGCTTGCCTCTTCATTGTAAT		TGATTCCTTTCTCTGCTCCATGTC
266	549	AAGCAGCGGCAATTTCACTTC		ATATACATCCAGATGCGCTCGAGTAA	P	GTGGAAATGTCATCAGTGAAGCCAGGA
267	550	CATGTTTTTTTAAATGCACTATCCA	P	AGAGACAAGAAATCACTTTGAAATATA	P	TTATCTTATTAATCTATGATTTATTAATATTAATATTAAT
268	551	TAGCTTTCAAAATCTACGCCCC	P	CAAAAGTGCAAAAAACAGATTCAGC		ACCCCAAATGCTAGTATTTCTTCCA
269	553	AGGAATGTATCCCTCAGCTCAC	P	AGGCAATGCTTAGTACTGGTGT		TCTCTGCCCATATTCGAAANAAT
270	554	GCCAAATCTGATTTCCCAAGATTT	P	AGAGAGAGTACAGTACGTTAATAATAG		ACGTGTAAATAATTAAGXKTAATAAGATA
271	555	TTTAAACATCTCGCAAGTTTCTGA	P	CATTTGTAACTGCCCATCTGG		TGTTGCCCTGCTCTTTTGCTTAC
272	556	AAGCAGTCCMAAACCTTGAGC		GATCTTGCAGCCCTTTTCCA	P	ACGTGAAGTTGTAAGAACTGACCTTTG
273	557	ATAAGCTGACTCTCTCTCATTTCTG	P	AATGGTACAGCACTTTAGCTATACCAA		CATACCAACCTCCAGAAACAAA
274	558	TAGGCACTTAGCTCTCTCTCCCT	P	GATGTTATAGACATGACAGAGATT		TCATGGCAGTGAATTTTCAGACAAATTA
275	559	AATCTCTGAATCTCGAGCCTTTCT	P	CTAACACCGTGTGTGTGTGTG		GTGTGTGTGTGTGTGTGTGTGTG
276	560	CTCCTGTACTCTCGACACTCAGA	P	TCAGATAAGTACTACACAACAATAAATAAGG		ATTAAATAAGGAATXKTAATAAGATA
277	561	GAGAAATCTCTCCTCTCAGAA		GACCATATGAATTAATCAAAATGAGGA	P	TCCTTTTAAAGGCTTTTACCTGAT
278	562	CAGCTCTCTCTCCCTGTC	P	TGACCTTGTCAATTTCTCCATCT		CAAGTATTTTTTTTTTTTGTTTTTT
279	563	GAATTCCTAATTACTTTGGCTATCAGC	P	TCAGAGTCCAGAGGGG	P	ATTTGTGATGTTAAAGATCTCTCTA
280	565	GTACATCCGAGAGGATAAGGG	P	TTTATGTAGGGCTTAATTAGGATTTCTATA		TTTCAATAACAGATGTAAAGATTTCTT
281	566	TTACTCTGTCTCTCTCTGATTTGAT	P	CAGAGCTGCCATTTTCAGC		CAATHAACCGCTCTTGACCTTTCT
282	567	AAATTTACTATGTGAATCTGGAAATC	P	TCGTGCTTAAGCATCTGCGCT		CAAGATTTTTTTTTTATTTGTTTCCAC
283	568	GGGCTCTATTCTTAGCAGACA		TGTTGTTTTGTTAAGAACTCTATCTCC	P	GGAACATAGTAGGCGCCAGTTTGAATGATGATCGAATGGGATGA
284	569	TTCCCGAGATGGAAATTTGT	P	GACACAGATAGGATGATGGCC	P	CTCCGACGATTTGATATAGAAACAC
285	570	GGACTTGCATAGCAAAATCAAC		TGTCAGTTTTAGTCACTCATATATACCT	P	ATGACTAGGAAAACATTTTGTAAAT
286	571	AAATCCCTCTCTTAAGCCATAGT	P	GAATGAACAGACTTTAGTGTTCAGAC		CTAAATGTGTGAGAAAACAGACGCCACATAGTTTCAGGCGCTGGCT
287	572	GTTTGCTCTGATCTCTCTTGG	P	TGAAGAAGTCTCCAAATTTGGAATATAT		GGTTTTCTACTAAATTTTAAAGTGTAGACTGGGCGCAAGCAATATAT
288	573	TAATATTGATCTCGAATATTGACAGG		TGCAATGAAGATTTGTAGATGAAATGA		TAAAGTGAGATAAGTGTGGTCCGTTTTCTGCGCCCAACCCCTCTCATTTTC
289	574	CACTTTAGAGATAGGAATTTTACTATAGTTAGA	P	TTCTTAAGAGCAATTTGGGTACTAGATTCT		AAACCTTAAATTTACTATAGAGAGACCTGATGAATATTAATCTTANAGCA
290	575	TTATTTTGTATAGGATATAAGACCACTTAATCA		TGATGCCCACTGCGG	P	CATGTTTGGCGGCGATGTTGGTCTCAAGCTGTAAATCCGACACTTTGGGAG
291	576	CAGCAGCACTGCTCTCTGTAC	P	ATAAGCAATGCCCTGACACTGGG	P	CGCTGTAAAAATGTGACGCCAATTA
292	577	TAGCCCTCTCTACTCTAGCCCACTC		CAAGAGTTACTGTGGTGTGTTTTTC		AGCTATAAAGCTCAAT

[illegible]

Row#	REF#	Upper PCR primer	1	2	Lower PCR primer	Modified	3	gBA primer	Modified	gBA primer	3	Flanking sequence
344	634	AGGACTAAACCCACATTTGGG			CTTGACTAGTCCCAAGTCGAAATTT		P	CCCAGTCATGGGGTGATTTGCTCTTGTGAGCCGACAGAAATGATGTGTTT				CCCAGTCATGGGGTGATTTGCTCTTGTGAGCCGACAGAAATGATGTGTTT
345	635	AACACAGACAGACCCCAACCC			CNACCCCTTTCTGATTTTCCC		P	CACAGCCACTGGCTTCTGTTCACTC				CACAGCCACTGGCTTCTGTTCACTC
346	636	TGCAGACTCCCGCCGAGG			CCGAGTGTGTTGAACTGGG		P	GGGTTGTGTGACTCAAGXCTGGGCT				CCAGCTCCAGCTGGGATTTGGACAGAGCCGAGGCTGTGACCAAC
347	637	CAACAGACATGATATTTGCA			TGGCTTAGAAGTCAAGAACAGG		P	CTGCTTAGTAAATATCTCTCTAT				TTGCTTTTGGTTTTCAAGCTTAATAGTAGAGATTAATTTAACTACAGTCA
348	638	TGACCCCTACAGACACTTAGTTTG			GTGATGGTGGCTGAGCTAGGATT		P	ATTTAGGACTATCAATCCCTATGTC				AGAGGCGATTTCTTAGAGTGGAAAGGACATAGGAGATTTGATAGTCTCTAAAT
349	639	GCCTTCCACTTTTGGACTGAT			AGAGCTTGGCTTAAAAATCCAC		P	ATGTGAGAGAAATTCCTAAATTTACAC				GTGAGAGAAATTCCTAAATTTACCACTCTTGGTGTCCCATCTCTGTG
350	641	TTAAAGTGGCGCTGCTGTATAC			GATTCAGGCCATGAGCCACC		P	CCAGCGXTTTAGTCTCAGGTAAACCA				AGCGTTTTTAGTCTCAGGTAAACCGACTCTTGGTGTCCCATCTCTGTGTTCA
351	643	TTAGTGTTTACAGAGTCTGGAGTG			TCTATGAGTCTCAAGTCTCTGATATG		P	TTAAAGTATGTGGGAGAAATCGGGTGTGTTATATATGCAAAATCTGTGTAAGGG				TTAAAGTATGTGGGAGAAATCGGGTGTGTTATATATGCAAAATCTGTGTAAGGG
352	644	TTTTTTTTTTTTTGACAGAGAGCT			CCGAGAGATCACTTGAACCC		P	AXCCCGAGGACAGAGGTTXAGTGAAG				CTCTGTCAACCGAGCTGGTGATCTCGGCTCACTCAACCACTCTCGCTCCGAGG
353	645	ATCCGCCCTCTGTGTCAACC			GGTACAGGCAAGATATAGAG		P	GCCGAGGAGTGGGATGTGTAGTGA				CTCGAGTGGAGTGGGATGTGTAGTGAATCAACCACTCTCAACCTCTCGCTCCGAGG
354	646	TTGGGGGAGGAGGACCT			CTCATGGAGAACTCCGCTATATG		P	AGGATTAAGTGGATGCTGCTCGTGT				CAGCACTGCTCTCCAGGCAATTCAGCAGGCAATTCAGCACTCTCTCTCTCTCT
355	647	GAAACCTCGCAATAGACACT			CTCATCTCCCACTCTGGC		P	TTAAATGTCTACCGTATATATGATG				GAGTCTGAAATTTATTTCAACGATATACATATATATATAGGTAGCATTTTAA
356	648	CCCACTCGAAGGTGACC			CNATTAACCTGACACCGCC		P	CAAGAGACTGGCGCAACACCGAGCA				CAACAGACTGGCGCAACACCGAGCAACCGAGGAGATTCAGAGGCTGTGTGT
357	650	AGTCTCTGCTTGGGCTTT			GGGTTAGAGCCGCAATATATG		P	CACTGAGCAAGTATTTCCCAACACCGAGCAACTCTTCAAGAGCTCCGAGTGT				CACTGAGCAAGTATTTCCCAACACCGAGCAACTCTTCAAGAGCTCCGAGTGT
358	651	TTGTGTGGGACAGCTGAGC			CGTGAAGGAGGCTGTGATTT		P	ACNACAGCAGATCAATTCGCCAACC				CCCAAGCTCTCTCGCAGCATCAAGGCTGACATTTGATGTGTGTCATGT
359	652	TGCTCGAAGATCCAAACATTT			TGAGATCCCCAAATCCAA		P	TCTGAATTTAGACTCTCTCCATGA				ACCAAAATGATTTCCAAAGGAGTCTCATTTGGAGGAGTCTAAATTTTCAGA
360	653	GTCCAAATTAATCTAAGCTATCCACTT			AGGACCTAACTAGGTTTGTCTACAG		P	ACNAGATGCAGTTTTGAATATAAT				CCCTCTACTTTTCATCAAGCTGACTCATATATATATCAAAAGTGCATCTGTTGT
361	936	CTCATGAACOMAGTATGAGCAATATTT			TCAGTGTAGTGGCTCTAATTAAGAT		P	TGCTGCAATTCGGGCAAACTCAGAAACG				TCGCAATTCGGGCAAACTCAGAAACG
362	937	TCATTTTCCATATGGCTACATATAC			AAATATAAGCTATGGCTATGAGCACTT		P	TTAAGACCAAGATTTCTCAGAGCAAC				CCATGAATCCAGACTCGAACAGATCTTGGCTTAGAGAGATTTGCGTGT
363	938	CTAGTCTGCAATTTTGGTGTA			CNATGAGTCTCATGATCCAGCC		P	GAAITCCCTAGGCGAGAAAGGAGCA				GAAITCCCTAGGCGAGAAAGGAGGATAGAGGACAGCATATCTTA
364	939	TCGCAAAATTTGGAACATAGCTG			GTATGTGCTTAAATCTCTCCAGA		P	GACTGTAAATGAGTTTTCTGTGTAG				GCTCTTAAATGAGTTTTCTGTGTAGGCAATTTTCTTACATGTGTGTCTAG
365	940	GCAACACAGCTGTGTGTATTAATTT			TCCMAATCCAGTATGACGAG		P	AACTGTGCATATAAGATTTXATAGAG				TTTATCCAGAGTGTGTGCGCAACTCTTCAATTAATTTCTTATATGCAACAG
366	941	TGAACCAAGCATTTGATATAGG			CCCAAAAGCCCAACAGGG		P	TGCAATGAGTTTTGCGCAAAATTTTC				TGCAATGAGTTTTGCGCAAAATTTTGTGTCGTGTAGGATTTTAGGT
367	942	TAGCTGGGATTCAGCGCC			GATCACTTCAGGCGAGGAAATTT		P	AAITTTTGATTTTAGTAGAGAGAGCTG				AAITTTTGATTTTAGTAGAGAGAGAGCTGTCGCACTTGTGGCGAGACTGG
368	943	ATTACAGAGTATTTCCAGGTGC			GTCAACAGCTGATGAGACATATATAC		P	TCTGTGGAAACAGAGAGAGCATCACTG				TCTGTGGAAACAGAGAGAGCATCACTGAGTGTGTGCAAGCGCTCTCTTAGG
369	944	TCCTGGATTTACAGGCTG			AACTTTTGGCTTAAAGAGCTGTG		P	CTGATTCACCAAGAGATTTAGAACG				CCCACTATCCCAAGAGATTTAGAACG
370	945	CTCCAGTATACAGAGGATATAGTCT			GTAAATGTCTGAGCTCAGATGGA		P	AAAGCCOMATACACAGCTAGCAG				AAAGCCOMATACACAGCTAGCAGCTCATTTCACTACTGCTCTCAACATG
371	946	ATCAGAGCACTTTGAGGCC			CAGCAAGCAGACTCTGTCTCTC		P	ACCACTCTTAACTAATGCTTAAACAA				ACCACTCTTAACTAATGCTTAAACAAAGGACAGACCCCATCTCTACAAACAA
372	947	CTCCATTTCTCGCAAAATG			TGCGTACTGTCTCTTTAGCATGA		P	AAAGATTTTGGAAAAATTTGACAGAT				AAAGATTTTGGAAAAATTTGACAGATTTTAAATATATATAGAAATAGA
373	948	ATATACAGCTGTGACATGATTAAGAA			CTTTTAGAGTAGTAGGGCTGCTC		P	GTGAGGCTGTGTGTCAGACAGACCC				GTCTTATCCAGATAGAGCCGTCAGAGGCGGT

Row#	REF#	Upper PCR primer	1	Modified	Lower PCR primer	2	Modified	3	Planking sequence	4
393	970	CAATCCCAATAGCTCTGCTC			GAATAATGTTGGAAAGGCA			P	GCTTCTTAGGCAACAACTTCTTTT	GCCTTTAGGCAACAACTTCTTTT
394	971	TGGAATATAGGAAATAGGTAATG			GGATCTTTCTGGYGCCA			P	AACAGTGGTTGAGGAGGAGGGGG	AACAAGTGGTTGAGGAGGAGGGGG
395	973	TGCTTGAAGTGTCTTCTGCTG			CTCAATATAGAGCTGTGTGGCC			P	CAGAGAGTCCAGGCACTTGGCCCA	CAGAGAGTCCAGGCACTTGGCCCA
396	975	GGCTTAGGCACTTGTCTG			ATAGCTGCTCTTCTGCTTAAAG			P	CTGCATCTACATGAGCTTAAAGATG	CTGCATCTACATGAGCTTAAAGATG
397	977	GTGAGTAATAACAGCAAGTGAATTTT			ATGTGCACTTACAGCTTAGGCCACA			P	AGCCGCACTTAAAGTATTTGCTGAC	AGCCGCACTTAAAGTATTTGCTGAC
398	978	ATTTCTCTTAGAGGTTCCCG			GATATTAAGCAAAATCTAGTAAGCACT			P	ATAACGCTTAAAGGCTGAGGAGG	ATAACGCTTAAAGGCTGAGGAGG
399	979	CAAGGAATATGATCCCTCACTCC			TGCTTAGTACTGTTGTGCTGAA			P	AATTCGACCCCTAGAAACCACTTAT	AATTCGACCCCTAGAAACCACTTAT
400	980	GTGCTAGAGAGGATGCA			TGCTCGAGGAAATATGTTCTG			P	AGAATATATAGGAACCACTTGA	AGAATATATAGGAACCACTTGA
401	981	ACTGAGCACTTCAATGCTATAT			CAGAGGAATGAGGACCTTAAAG			P	AGACTTTCACATGTTCTCTATGA	AGACTTTCACATGTTCTCTATGA
402	982	ACGAGTAGCAGCAATCTG			CAATACAAACAGTACAGAGTCTCT			P	TGCCAGCTCTTCCGCTTATTCACCC	TGCCAGCTCTTCCGCTTATTCACCC
403	983	CGAGATTCAGCAATATATACCTCAAT			TCATGCTTAAACATCAGCCTGTG			P	TGTGAATAGTAGTAGXGAATCTTGA	TGTGAATAGTAGTAGXGAATCTTGA
404	984	CAGAAATAGCACTGCTATGAGAGTAGC			GCATCAGCAACCTCCAGCA			P	AATATATATATGAATATTTGATGAT	AATATATATATGAATATTTGATGAT
405	985	TATATAGCACTGTTGGCCCAT			CAITTGATATGCTAGCCTGAGG			P	GATATATAGCACTGTTGGCCCAT	GATATATAGCACTGTTGGCCCAT
406	986	AAAGGTTTATTCAGGCAATATATAG			GTGTGCTGCTGCACAGC			P	TTATTCAGGCCCACTATATCTTCCA	TTATTCAGGCCCACTATATCTTCCA
407	987	TGAGTGTCTATTTGCTGCTCT			GATACTATTAGAGTGTGTAGGAACGTAATTA			P	CGATCTGCACTGTTGCTGCTGCT	CGATCTGCACTGTTGCTGCTGCT
408	988	CTGAGCACTGCTGCTCTCT			AGGAGAAACGCTATGTAAGCACTTATAGTA			P	TCTTAGAATTCAGAAATATTAATG	TCTTAGAATTCAGAAATATTAATG
409	989	CTGAGTGTGCTGCTCTCT			CACAGCTATTCTGCTAGCTTTTATTT			P	TTTGGGCAAGCTGCGAATTCGAA	TTTGGGCAAGCTGCGAATTCGAA
410	990	ATTCGACCTCAATGCACTG			CACTGCTCGCAACAGATGCTGATG			P	CAAGACCTCAGTATCTGCTTCAAGT	CAAGACCTCAGTATCTGCTTCAAGT
411	991	AGCGCACTGCTGCTGCA			CTTTCAGAGCAGATCTGCG			P	GGGCTGAGGAGGAAATGCGT	GGGCTGAGGAGGAAATGCGT
412	992	AACAGAGTACAGCTGCTGCTG			CAACTGAGGCGCTTCA			P	AAAGCAAACTAGGCTGAAATAG	AAAGCAAACTAGGCTGAAATAG
413	994	ATCCCTCTCTGCTGCA			GGTACAGGAGGATGAGG			P	GCGGTGAGGAGGAAATGCGT	GCGGTGAGGAGGAAATGCGT
414	995	TGGGCGAGGAGGAGGAGG			CTCAATGGAATGCTGCTGTAAG			P	AGGATTAAGTGAAGTGTGCTGCT	AGGATTAAGTGAAGTGTGCTGCT
415	996	CCAGCTGAGGAGGAGGAGG			CAATTAAGCTGCAAGCAGC			P	CAGAGACTGGGCAACAGGAGG	CAGAGACTGGGCAACAGGAGG
416	997	CGAGGAGTCTGCTGCA			GTTCAGCCTGCTGCTG			P	CTGCGAGCGGAGGTTACAGTACG	CTGCGAGCGGAGGTTACAGTACG
417	998	CTGCTGAAATTAAGTGTGAGCAATGA			CTTTTATTAAGAGCAGAGTAAATGTTGATAC			P	CACTTCCATATGCTTCTTCTGCT	CACTTCCATATGCTTCTTCTGCT
418	1000	CTCTCTTAATTTCTTCTTGTAGCA			ACTGAGGCTGCAATGAC			P	CATGCAATACATCATTTACTAAT	CATGCAATACATCATTTACTAAT
419	1001	TCCCGACAGATGTGAAATG			CAITTTATAGATTTCTAGCAATG			P	GCTGAACCTCTTCCCTTTATAAAT	GCTGAACCTCTTCCCTTTATAAAT
420	1002	AAATGCTGCTGCTGAAAGG			CAATCGGAGTCTGCAAT			P	AACTGCTTCAATCTGCTGCAATTA	AACTGCTTCAATCTGCTGCAATTA
421	1003	CAAGAGTAGGAGTATGCTAT			CCTCATCTTCTGCTTCTTCTAAGC			P	AGGATTAAGTGAAGTGTGCTGCT	AGGATTAAGTGAAGTGTGCTGCT
422	1004	CAATCACTGCTGCTGCTCA			GATTAATCAGAGAGGATGCTGCA			P	CAAGAGACTGGGCAACAGGAGG	CAAGAGACTGGGCAACAGGAGG
423	1005	CAATTTCTGAGTCTGCTGCTCA			TTTAGATAAATCCAAACTCTTCTCTC			P	CACTTCCATATGCTTCTTCTGCT	CACTTCCATATGCTTCTTCTGCT
424	1006	TAAATTAACATGAGTCTTCCAGTGC			GACAAATGACTTACAGATCCGCC			P	CTGCGAGCGGAGGTTACAGTACG	CTGCGAGCGGAGGTTACAGTACG
425	1008	AAITGCTGATGCAAGAGAA			GCATAATGATATGGAATTTTCAAGATCA			P	AGATTAAGTGAAGTGTGCTGCT	AGATTAAGTGAAGTGTGCTGCT
426	1009	AGGTGCTGAGGAGGAGTGG			GTGAGGCTCTGAGAGAC			P	GAAGTAGAAGTGCATTTGGAATG	GAAGTAGAAGTGCATTTGGAATG
427	1010	TTCAGGCTGAGGAGGAGG			CCTAAGAGTTTTTCTAGTGTACAGCC			P	AAAGAGTGAACATCAGAAACAG	AAAGAGTGAACATCAGAAACAG
428	1011	TACAGTGGAGGTTTAAACATC			CTAGCTCTGAGGAGC			P	AGAGTGAAGTGTGCTTACCT	AGAGTGAAGTGTGCTTACCT
429	1012	TGAGTGCATAGGCTGA			ACATTAAGCAATGAGACTTAAACAA			P	GATGCAAGTGGAGAACCTCAGG	GATGCAAGTGGAGAACCTCAGG
430	1013	TGAGTGCATAGGAGTATTCATG			CAGAACAGAGAGGTTGGTG			P	GAATGCAAGTGGAGAACCTCAGG	GAATGCAAGTGGAGAACCTCAGG
431	1014	AAATTTTCCAGCAATACCTTTT			CAAAAGCTTCCGAGGCTGG			P	AAAGAGTGAACATCAGAAACAG	AAAGAGTGAACATCAGAAACAG
432	1015	TCTGAGTCTGAGTCTTCTG			CTCTAGTCTGCAATATTAGCAATGCTG			P	GAATGCAAGTGGAGAACCTCAGG	GAATGCAAGTGGAGAACCTCAGG
433	1016	TCTGAGTCTGAGTCTTCTG			GACTTACCTGCTGCTGCTATCA			P	GAATGCAAGTGGAGAACCTCAGG	GAATGCAAGTGGAGAACCTCAGG
434	1017	TTCGAACTGCAATAGGAGG			CAGCCTACCTGCTGCTG			P	GAATGCAAGTGGAGAACCTCAGG	GAATGCAAGTGGAGAACCTCAGG
435	1018	ATGCACTGCTGCTGCTG			GTTCATCTGCTGCTGCTGCTG			P	GAATGCAAGTGGAGAACCTCAGG	GAATGCAAGTGGAGAACCTCAGG
436	1019	CTGCTGCTGCTGCTGCTG			CTTTTAGGAGTACATGCTGAGC			P	GAATGCAAGTGGAGAACCTCAGG	GAATGCAAGTGGAGAACCTCAGG
437	1020	ACTGAGCACTACAGATTTTCAACA			AGCTCATTTTGGAGTATGATGTA			P	GAATGCAAGTGGAGAACCTCAGG	GAATGCAAGTGGAGAACCTCAGG
438	1021	AAATGCTGCTGCTGCTGCTG			TTATTTGAGAGGCTGCTGCTGCTG			P	GAATGCAAGTGGAGAACCTCAGG	GAATGCAAGTGGAGAACCTCAGG
439	1022	AGACACACAGCAATAGACAGATAG			TTCGCTTGGAAAGTATGAGC			P	GAATGCAAGTGGAGAACCTCAGG	GAATGCAAGTGGAGAACCTCAGG
440	1023	GGTACGAGGATGCTGCTG			TCCAGGCAATTAATAATCAAGTGA			P	GAATGCAAGTGGAGAACCTCAGG	GAATGCAAGTGGAGAACCTCAGG
441	1024	GCAATCTCTTCTTCTATGAGAGTGA						P	GAATGCAAGTGGAGAACCTCAGG	GAATGCAAGTGGAGAACCTCAGG

[illegible]

Row#	REF#	Upper PCR primer	1	2	3	Flanking sequence
		Lower PCR primer				
491	1078	GTTCCTGGACTAAATGATTTTTCAAA	P	ACGATAAGAGATGGCATAGATTC	GATTGAAAGATTTTTTTTTTCTGTAA	TTAGTTCTCTTTGTGATATTACAGCTTACAGAAAAAATAAATCTCTTGAACT
492	1079	TGGTGAATCAGCTCTGCAT	P	CTGACTTAATTTTGTATTTTGTAGTAAATGG	TGCAACTCTGCTGACTCTCAAGTCAATTC	CAACACTTTGGGAGGCGCTAGCTGGGCGCAATCATCTTACGAGTCAGAGATTTGCA
493	1080	AAAAACAACACGCGCTCCACA	P	GAAAGATTAAGGAATACTGCTTTGG	GAACTTTGCGATCAGACACACCCAC	TTCCGAATGCTCTTGACACAGCTTCGTGGTGGTGTGTGTGATCTGCAATGCTTC
494	1081	CTCTCTAGAAATCTGTTAAATGCTTCT	P	AGTTCTTAATTTCCACTCTGAGCC	GATCAATTAAGCTATGATGATTCGATGAGAC	TTAAAAATGCTGATGATGATACCAATGAATTTACAGATCTGAGAGTCTCTC
495	1082	TGAATACACACACACATGAC	P	CTTAGACCGGCTCGGAG	TTCCACACACGACGGAAGAGGAGAC	TTCCACACCGGAAAGAGGAGACGACGACACGAGCGCGCACCGGCGCTCCGA
496	1083	CTATCATGCACTGACATATGCTGTAA	P	TTAGACAAGTGTAAATTTATAGAAAGAAAGTGA	CTTGGTATAGCTGCTCTCTTAAATATGG	TGTTATATGCTTTTAAAGATGGGGTAAATTTATATATGATGATCTAAATTAAC
497	1085	GAGAGGCACTAAACAGACACTTAAATTT	P	TCCTTAGCGCTCCAGCTG	AGAAATTTAATTCATTTTCTTTTATTTA	AGTATTAAGCTCTMACTTTTAAAGATTTTTATAGTTTTATAGTTTTATAGATTT
498	1086	GGACATGGATATCTTTTCATTTT	P	AGCAACGAAATTAAGTACTTAGGAAAAA	AGTTCTCTTTCTTTCTTTCTTATG	AGTTCTCTTTCTTTCTTTCTTATCAGAGATTTATAGTTTTTCTAGGATTT
499	1087	ATTGTGATTTGATTCGATCTAGACTCT	P	GGAGAGCTAGCTGGGGTGG	TCTCTTCTGGTGACGCTGTGGCTTCG	TCTTCTGCTGACCATGTTGCTTTCCCTCTCTAGGCTCTTCCGAGCCCGCA
500	1088	TCAGTCCGAGCTAGCTTGG	P	CTTGAGTACAGTCACTGATGATATGG	GTGAGTGGGAAGATGATGATTAAGCCT	GGAGGTGGGAGATGATTAAGCTCTAGAGGATGAGGCTCTGAGTGAAGCAT
501	1089	GCATTCGCGAGTTTCATTTATGTT	P	AAAATTCAGCTTTAGCCGATCA	ATGGAATAAAGTGTGTAGCTCTACA	GAGAAAAAAGTGTTAGACTCTACAGCTAGAAATAAATTTAGTAACTGAGCT
502	1090	TTCCCTGTACCCACCCC	P	CATAAAGCAGAATGTGTGTGTG	GCTCACTGGXAAAGATXCTGTGGAACAT	TGCTCAAACTGTTCAACAATCTCTCATGTTTCCAGAGATCTTTTCCGAGTGA
503	1091	CAGCATTCGGTCAGAGCA	P	TAAAGTTTGGGCAATTAACCA	CANAAGTAGCTGTGGCTGAACACA	CANAAGTAGCTGTGGCTGAACAACAGAGATTCAATGTGTACTCTTTGTGACTGA
504	1092	CTCTGTTTCTGGGCTCGACA	P	TTTTTCTGTTTGTATATGTAGAAAGTG	TGTATACCCCTTAAGCAAGTATTTT	TGATACCCCTTAAGCAAGTCAATTTTGGCCATTTTGGCCATCTGAGTTCTACAT
505	1093	CGGTACCGAGGATCTG	P	TTCTTGCTTTTGTAGTCTAGGAACTA	CAAACTCTGCTGTTTCTAAGTGAGC	AATCAGCTGCTTTCTAAGTGAACGGTATATACCTTTATGTTTATGTAGTTTCC
506	1094	TTATATATAGGAAGCGCCCTGGT	P	CTTGTACAGCTTTAGGCT	CTGTCTCTGGGATTTCTCTCATTC	CTGTCTCTGGGATTTCTCTCATTTGTAACCTCTCTGTTCTATCATGCTGACG
507	1095	TAGTCCACAGCGGCTAGAA	P	CTCTTAAGTGTCTCCCTGGG	TXTCCTCTGAAATAGAAAXCTCTAGT	ACCAAAACAGCTTCAATCAAGCTAAGACTAGAGCTTTCTAAATTTACAGAGG
508	1096	CAACAGAGATTTCAACAGAGTT	P	CAGCGTGGTCTTCTATCA	CCTGGCCACTCTCCAGATAGTCA	AAGAGCCTCAACGTTTCAAGAGCTCACTATCTTCGCGAGAGTGGCAGG
509	1097	CTTCTCATTTGATACATTTTTTTTTTCC	P	CAGCGCAATTTCTGAGCC	CXAGCTGGGTGAGTGGTGGCATCT	AGCTGGGTGAGTGGTGGCATCTCGGCTCACTCAACCACTCTACTCTCCCG
510	1098	TACCGGGATCTTATCAATAGTCA	P	CATGAGACCTGGCAGGAAA	CTTTGGTCTGACCTGAGAGTCAACC	GAGTCCAGCTCCAGGTTGAAGTCTCGGCTATGATCTCTCCAGGTCAGACCAA
511	1099	TCAGTCTCAGACCAAGGCA	P	ATTATACCTCTGACTGCAACCTC	ACACTAGCTTGGGGAGGACAGACT	TTTCTCTGAGGTCCTATGAGAGAGTCTGCTCTCCCAACATCTGCTAGTGT
512	1100	CTTGAGATTTCAATTTTTTCTCTCTC	P	TTTTATCTGGGTTTCTAAATATTGC	TAAATATCGCTGAACGTGATTAACAT	CTTCAAGAAGAGCCCATATGCGACAGTGAATTAATCAGTTTACTGCAATAAT
513	1101	ATTAGAAATCTCTCCGACG	P	GTTTTGTCACTGTCTCAACATG	TCTCCTTTCTCTTAXCAGCTCTGATC	GGACTATCATCTCTCTCTCTCTCGAGTTTGTGTTCTCTCCCTTGGTTTTTC
514	1102	GCATCCGAGACATCTC	P	TTTTATCTAGAAATCTCTCTTCC	CAGTGTAAAGGAGTGTGGTGG	AGAGATAGCTTTAACATATCTAAAGAATCAAGACTGATAGAGAAAGAGAG
515	1103	GGGGATCTTTCTCTGTTATTA	P	GTGTTAGACTGTGGGACGCT	CACTGTAAAGGAGTGTGGTGG	TTTGTAGGCTSTGACACTAAGATCGCCCAACAACTCAACCTTAAACAGTGT
516	1104	TGCTCAGGCCCTTAAGT	P	TTCAAGTTCACTTGCCGACATTT	TCCTGXATATAGCACGACGACACC	TCGGCATTTAGCCACGACGACCGCTCTCAACAACAACATTCATATTA
517	1105	AACTGAAGACACGACG	P	CTTCCACAGAGTGTACCCAC	AGCACCTGAGCTCTCCACAGAC	AGCACCTGAGCTCTCCACAGAGCTTTAAGCCCAAGGATTCAGAGGTG
518	1106	CTGACTTTCTGAGGATTAACAA	P	AGGTCATATGAGATATGCA	TTTTAAITTCATGAGTTATTTGG	CAAAACAAATAATTAACAGAAAAACCAAATAACTTTCAAAGATTA
519	1107	ATCTCAGCATCAAGAGACTTGTCTC	P	TACTCTCGCTCTCCYAAGTCT	AGTCTGGGATTAACAGATGAGCGAC	ATAATCAGAGCTCTGGCGAGGCGGCTCGCTCATCCGTGTATATCCGAC
520	1108	CTAGAGTGTGTTCAAGACTAAATGCT	P	TTTCTCTGATCTGTAGGCTCAAA	TCCTATCGGGCTTAAGCTGAAATAG	TCCTATCGGGCTTAAGCTGAAATAGGTTTCGACCTCAACGATCTAGTTTG
521	1109	GTTGTGTGTTTCTAAGATTTGG	P	ACCGAATTAATCTTAACGAGAAGGA	AGCAAGCAAAAGCGCATTA	TAATACATTTTTAAAAATAGATCCGTTTTTAATGCGCTCTTTTCTCTCT
522	1110	CTGACATTAAGAGCTCCACATTT	P	TTGTGCTCTGGTTTCTCAATAATTT	ATAATTTGGTCTTATTTCTTAACAT	AGCATGCAACAAAAAATTAACAGGCTATCCAGGCTTTTACTTCAACGATTA
523	1111	CTTTTCACACACAGGCG	P	AAGAGATAATAGGAATGTCTATGGAA	TTATGGGAATTTGAGAGTANAAGG	AAACCATATCAGATTTTTTAAAAATCCCTTTTACTTCTCAACATTTCCCATCAT
524	1112	ACAGTTTGAATTTATGGGAC	P	CATGTGCTCTTGAAGGGCT	GTAGCATTTGGATGGGGACACGCA	AAGATTTGGAATGGGACACACGCAACCATATCAACCTCCCATTTGAGCTTACT
525	1113	ATTCCGCGCATCTA	P	TACATTCAGCTCGGCA	AAAAAATAAAGTAAAGACATACCC	CTTTCTTTATAAATACCCAGCTCTCGGGTATGTTCTTTAGCTTTTATTTTTT
526	1114	GTGGGCTCAATACTCATGCT	P	TGGTAGCTGGGATCC	AGATTTATGATTTATCTATCTATAGGT	TTCCAGAGCTTTTATGATCATCTACGCTTACGCTTATAGGATTAATCAGATTAAT
527	1115	GAGCTATCTATCAATCCACAGA	P	TCGCTGAGCTGGGATCC	CATCATACGAAATAAACTCTCGAAG	CTCATGAACGAATAAAATCTGGAAGCATATATACTTCTGATACTATATAAGA

Row#	REF#	Upper PCR primer	1	Modified	Lower PCR primer	2	Modified	3	GBA primer	Planking sequence	4
540	1131	ATTAAAGTGCAGTGGGAGAGCT			TTATTGTCTATCTCTCTCCATTAATAAAG			TTTAGAGTGCXGCTGTGTGXCAAGCA	P	TAGAGTGCCTGCTGTGTGCCAGGAGCTTTCTAGATGCGAGTAGGTATAGCTC	
541	1132	ACATATGCTGCTGCTACCC			ATGATCTTTCTTAATGCTTTCCATGAA			AAAGACTTTAGACAGCCACACATAA	P	AAAGACTTTAGACAGCCACACATAA	
542	1133	GTAGAGTGCCTTTATTTTCTTTT			TGCCATTTCTCCCCAAA			CAATTTCTCAAAAACCAATCTG	P	CAATTTCTCAAAAACCAATCTG	
543	1134	GTTCATGCTTACATAGTAGTACGATGAT			TGCGAGTAGCTCTGTATGACATGC			TTTAAATAATGCTCTTCTACCTCT	P	TTTAAATAATGCTCTTCTACCTCT	
544	1135	CACTCTCACTTTTGTGATTTT			AGTTCCTAGCTTTGGGATACCTTTTAA			AAATACAGCATAGATACCAATAT	P	AAATACAGCATAGATACCAATAT	
545	1136	ATTTTTCGCTGCTATTTCTCTG			CAVTTGGGTAAATACCAAGCTTCA			AGAAATTCGCTAAATACCAAGGCTAC	P	AGAAATTCGCTAAATACCAAGGCTAC	
546	1137	GATTCCTAGGCGGAGG			ATAATTAACCAACCACTTAAACCAATTTA			CATATTAGTCTCTTCACTTAAAGCA	P	CATATTAGTCTCTTCACTTAAAGCA	
547	1138	ATCTCCACACCTCTCTGATC			GAAGAAGTGCACGAAATACGATTTAG			CGATCGATGACACAGCTTTGATGAC	P	CGATCGATGACACAGCTTTGATGAC	
548	1139	GATTTAGTGTATAGTATATAGTTATTTAGATG			TCATTAGTCTTAACTTTTGAATATGTC			AGCATATTTGATTTGCTACTTTAGCA	P	AGCATATTTGATTTGCTACTTTAGCA	
549	1140	CGCGGATCTGAAAGGCA			TAAATGTGCTGCAACCAAGG			ACAGATTAATTTGCTCTTTGGCTG	P	ACAGATTAATTTGCTCTTTGGCTG	
550	1141	TAACTGTAGCTCTTCCGAAATTC			CTCTCAATTAACAAACAGCAGCC			TGATTAATTCAGTCTTATGCTTGGCTG	P	TGATTAATTCAGTCTTATGCTTGGCTG	
551	1142	GACTCTGAGCTTGAAGAACCTCC			CTTCAATTAACAAACAGCAGCC			CCTCATGATCTGCTAACTCCACTTC	P	CCTCATGATCTGCTAACTCCACTTC	
552	1143	CGGTATGGAATTTCTTAAAGTATAGAA			TGCGACATAATGCTGGG			ACAGATTAATTTGCTCTTTGGCTG	P	ACAGATTAATTTGCTCTTTGGCTG	
553	1144	CAATTTCTGCTTAATCTGCTTTT			TAAAGTGCACCAATGAGAGTTCA			TGAATAATTCAGTCTTATGCTTGGCTG	P	TGAATAATTCAGTCTTATGCTTGGCTG	
554	1145	GCAACATCTATGCTGCTTTT			CCAGGGAATGCAATTAAGATG			TACCCAGGTTATTTTATTTTAGT	P	TACCCAGGTTATTTTATTTTAGT	
555	1146	AAGAGGAACTCTGCTAGCTTT			AGCTTGAAGTCTAGTACGAGG			CAGTGAGATACCACTTATTC	P	CAGTGAGATACCACTTATTC	
556	1148	ATCTAGAGCTCTGTAATGACTATCC			CAITCTAAATAGCTACTTTCCAGGG			AGAGTCCATCTGCTACTTTGATTTG	P	AGAGTCCATCTGCTACTTTGATTTG	
557	1149	AAATAATGCTGCTTCTGCTATAT			AGCTTGAAGTCTAGTACGAGG			AGCTGGCATAGGCTGATAGCTG	P	AGCTGGCATAGGCTGATAGCTG	
558	1150	GAGATATGCTTCTGCTATCTGCA			GATGAGTCCAGGCTGGCA			CAAGTCCATCTGCTACTTTGCTTTA	P	CAAGTCCATCTGCTACTTTGCTTTA	
559	1151	AAATGGGCAAGGATGG			CTTTCTCATGATCTTGGACTTCT			TCAGTACTTTCTCAGTCTCAGGCA	P	TCAGTACTTTCTCAGTCTCAGGCA	
560	1152	GATGCTGAGTATAGAGTCTATATATAC			TGGAATTTGTAATGCTGACTTG			AGGGCTTCTCAGTCTCAGGCA	P	AGGGCTTCTCAGTCTCAGGCA	
561	1153	TACGGGATCTGCTTAGC			CAAGTCCAGGATGAGAGTGG			TAGCAATTCACACAGGCA	P	TAGCAATTCACACAGGCA	
562	1154	ATCCAGCTATATCTCTATCTGAC			GACACAGGATGCTCAAAACCA			CTACACTGCTCTCTTACAGAACT	P	CTACACTGCTCTCTTACAGAACT	
563	1155	ATAGAGGCTGAGCTGTAGACCA			CAATACAGGAGATTTCTATGATTTT			CTGTGTGCTCTCAGCTCTTACAGT	P	CTGTGTGCTCTCAGCTCTTACAGT	
564	1156	TTATGCTTAAATCTGAGTCTGATGAC			GGGATCTGCTGAGTCTCTCT			CTATGAAATTAACAGAGTATGAA	P	CTATGAAATTAACAGAGTATGAA	
565	1157	ACTGCTTACCCAGGAGCATG			TAAATTTGCTGCTCTCTCT			AACTATGTTAAAGGCTTAAAGTCT	P	AACTATGTTAAAGGCTTAAAGTCT	
566	1158	TGGGTATAGTCAATCTGATAGGC			GTATTTAGGCAATTTCAAAAGTAGACCT			CTCATCTGCTGCTTTACAGTCTCT	P	CTCATCTGCTGCTTTACAGTCTCT	
567	1159	TACATGCTGATCTGAGAGATATTTGA			CGCTATAGAGATCTACAGAGATCC			ATATATTCTGCTCTTCTGATTTAT	P	ATATATTCTGCTCTTCTGATTTAT	
568	1160	CAAAAGCATTAAGTCTTATGTAAG			AGTCTGTTAAATGCTTAAATAGCTTG			TAAATAGCTCTGCTGCTCTCTCT	P	TAAATAGCTCTGCTGCTCTCTCT	
569	1161	CTACAAAGCAATGCTG			CCAGCCGCTGAACTG			TCCTCAGCTCTTAAAGAGCAT	P	TCCTCAGCTCTTAAAGAGCAT	
570	1162	CAAGATTAAAGAGAAATGCTTGAATAC			CTGCTGCCCTCTGAGAGAT			COATTTAGATTTGTAAGTTTCTGAG	P	COATTTAGATTTGTAAGTTTCTGAG	
571	1163	CGATCTTGGAGGCTG			TCCAGATGCAATCCACT			GAAAGCTGTTGGAATTTTCAANA	P	GAAAGCTGTTGGAATTTTCAANA	
572	1164	CTGATTTCTGCTTAAATG			CTGCTGCTGCTGCTG			TTTCCCAACCAAGAGATTAATA	P	TTTCCCAACCAAGAGATTAATA	
573	1165	CGGCACTCTGCTTAAATG			CTGCTGCTGCTGCTG			TGCTCTGCTGCTGCTGCTGCTG	P	TGCTCTGCTGCTGCTGCTGCTG	
574	1166	TACGCTGACAGAGATAGTATGG			CTGATATGTAATGCTGCTG			GAACTGCTGCTGCTGCTGCTG	P	GAACTGCTGCTGCTGCTGCTG	
575	1167	CAATCACTCTCCACAGCT			CTGATATGTAATGCTGCTG			TCCTCTGCTGCTGCTGCTGCTG	P	TCCTCTGCTGCTGCTGCTGCTG	
576	1168	TAATATCAAGAGGAGCTGCTGCTTC			CTGATATGTAATGCTGCTG			ATTCACTCTGAAACTTACCTTTG	P	ATTCACTCTGAAACTTACCTTTG	
577	1169	CTGGGACGCTGAGGTGA			AGGCGCATGATTTTCAAGTCTT			CTTCACTCTGCTGCTGCTGCTG	P	CTTCACTCTGCTGCTGCTGCTG	
578	1170	GATTCGCGAGATATCATCATATTATTA			AGAAATCTTTTCTCTCAGCC			ATTGATAGTGTGTTATTTAGCTG	P	ATTGATAGTGTGTTATTTAGCTG	
579	1171	ATAGCAAGTCACTTACTGCTGCTG			CAAGATGCTGAGAGCTGCTG			AGACCACTCTGCTGCTGCTGCTG	P	AGACCACTCTGCTGCTGCTGCTG	
580	1172	AGCAAGTCACTTACTGCTGCTG			CAAGATGCTGAGAGCTGCTG			TATTTAGTACAGATGCTGCTGCTG	P	TATTTAGTACAGATGCTGCTGCTG	
581	1173	AGCAAGTCACTTACTGCTGCTG			CAAGATGCTGAGAGCTGCTG			ACCTAAGGAGATGCTGCTGCTG	P	ACCTAAGGAGATGCTGCTGCTG	
582	1174	TATGCTGAGAGAGGCTGAGCA			CAAGATGCTGAGAGCTGCTG			AACTTTCAATTAATAGGAGG	P	AACTTTCAATTAATAGGAGG	
583	1175	CGATGATCTTCTGCTGCTGCTG			ATTGATGCTGCTGCTGCTGCTG			TTCCAGTCTGCTGCTGCTGCTG	P	TTCCAGTCTGCTGCTGCTGCTG	
584	1176	CGATGATCTTCTGCTGCTGCTG			CTGCTGCTGCTGCTGCTGCTG			CAAGAGCTGCTGCTGCTGCTGCTG	P	CAAGAGCTGCTGCTGCTGCTGCTG	
585	1177	CGATGATCTTCTGCTGCTGCTG			CTGCTGCTGCTGCTGCTGCTG			CAAGAGCTGCTGCTGCTGCTGCTG	P	CAAGAGCTGCTGCTGCTGCTGCTG	
586	1178	AGGCAATCTCCCACTG			CTGCTGCTGCTGCTGCTGCTG			TGTAATGCTGCTGCTGCTGCTGCTG	P	TGTAATGCTGCTGCTGCTGCTGCTG	
587	1179	CAAGAGCTGCTGCTGCTGCTG			TGCTGCTGCTGCTGCTGCTGCTG			CTAGATAACATCTTCTGCTGCTGCTG	P	CTAGATAACATCTTCTGCTGCTGCTG	
588	1181	AACTGCTGCTGCTGCTGCTGCTG			CTGCTGCTGCTGCTGCTGCTGCTG			AGCTCTGCTGCTGCTGCTGCTGCTG	P	AGCTCTGCTGCTGCTGCTGCTGCTG	

[illegible]

[illegible]

Row#	REF#	Upper PCR primer	1	2	3	Modified csa primer	Flanking sequence
687	1284	CACAATCAGGTCGTATTTTACAAA	P	TGCATTACACTAGTATCAGAGAAATACAGAA	P	AGCATGCTGCTGTTTGGXATTCCTCATG	AACCTTGAANAATACTTCTAAGCTAGGCAATGGGAATGGGAATTTTCAAAACAGACAATTCCT
688	1285	TATGTGACTTGGCTGTATCTCAGAC	P	CATTTCTCTGTAAATTTTCACTCTCCTC	P	AATGCTCAGGCXTTTAGGACGTAGATAT	CNAACCTCGAGGTTCTTAACTACTGATCATATCTAGTCTCTTAAGGGCTGAGACA
689	1286	TGTATATCGGTGACAGAGGCTAGTCT	P	ATGCATTTGGAAATTAAGGTAGTCTCTT	P	TAGTCTCTTAAANAATTAATAAAAAAG	CTGCAAAATTTAAATTTTCCGATACTTCTTTTCTTTTCTTTTAAAGAGACTA
690	1287	CACCTTCTGTGACAGACCC	P	GTCATCAGCTGGGGTG	P	GGTGTGAGCCGCTCTGCTCGAGAGT	AAACCTCGGCCCTCTATCTTAACTACTTCTTCTCTCAGACAGGGGCTCAACACC
691	1288	ATGAATGAATGACGAGTAGGAG	P	CAGATAATCCCGACAGACTCAGACC	P	CCCTGTGACXAGGTGCTTTGGGGACAG	CTGTGACAGAGGTGGTGTGGGACAGACGCCCTCTCAGATCAGCCCCAGGCTGTG
692	1289	CCGCTCTGTATGCTCTCTAAAAA	P	GGGATCAGATTTAAAGACATAGATAAGTATATGC	P	AGTATATGTCAGCAGAGTCTATAGACGC	TTTCTATTCGGCATCGAATAITATAGATGGCGCTCTATACACTCTGCTGCTATACT
693	1291	AATGACGCCCACTATACGAAA	P	CATCAGAACCCCAAGAACCTG	P	CTCTCXACGCTGAGAGTCTCTGTGGG	AAAGTTCAGGAGGGCTTTCTCCTGGGGCCCAACAGACACTCTCAAGGGGAG
694	1292	AAATCTGATTTGAATATGACTCCGA	P	CATCGAGATCTGTGTCAGGATGTTTCA	P	CTCATCTGTCTAGTTTACTCTCAACCA	ATCCCATATGTCATTCGGATTAAGCTGACGACAGCTCTATACAGTCAATCATGAG
695	1293	AAATCTGAGGTTCCCTCCCA	P	CTAAATCGGGAAGACAGCAGAG	P	AGAGCAGCAGGCTGGCGGGACAGC	CTAGTCTCTGCACATCTCAGCTCAGCGGTGTGCGCCGACGCTTGCTCTCT
696	1294	AGCTTATCCAAATGACTCTCTCGAAATAA	P	GGGGCATATGTTTATGAGGATTTCT	P	CACTAAACCTCTTATCTATATGTTGGCC	CTAAACCTCTTATCTATATGTCGGCGCACTTGGCCACACAGACATAGGGAG
697	1295	ACAGAAATTAATCTGTGCATCATATGG	P	TAATAATATGTATGTTTATCTCTGACTGTC	P	GAGACAGGAAACCAACCATGCCCC	GAGAGCAGGAAGTTTTTCAGGGTGTTTAGCAGCAAGGTTGGAGAACTGACGAGG
698	1296	AATCAAAAAGAGGAGGAGAAAGAA	P	CTTAAATATTTCTAGCCCTCTGTCTC	P	TGAGGAGAGTTTTTCAGGGTTTA	TCAGGAGAAATTTTTTCAGGGTGTTTAGCAGCAAGGTTGGAGAACTGACGAGG
699	1297	ACTTTGTTTCTGCCAATCGCA	P	GAGCTGATGATTCGAAATCTCTC	P	AAACGTCGAACCCCACTGACTTACT	CAAGACAAAACAGATAGGAGAACACAGCTAAGTCTCAGGCTGGGTTTCAGGTTCT
700	1298	TATCATATATAATATATACCCAAACAGACAG	P	CATGAGAGTCCCTGAGCTCCAGAC	P	CCACAGATTAATGGGGAGAGTAGAC	GCCAGGAGATCAAGGAGGAGAGACAGCTCCGACAGACAGACAAGGAGTCTCAG
701	1299	GGGACAGCGGAGGAGACT	P	AGGCTGTGTTTAAATAGCTCGA	P	GCACGAGTAACGAGCGGAGAGAC	GGACACTGGGCTTTTGGAGTCGCGGCTTCGGCGGGACATATGGACTCGAGAG
702	1300	TATCTACTCAGAAACGACGACA	P	CTTGCAGGACAGTGGCTT	P	CTCTCGAGTCATGTCCTCCCGCCAG	AGAAAAATTCAAATGTACTAAGAGTCCGCAACCACTCTCAGACGCTCTAGAA
703	1301	TGTTCCAGACATAAAGATCAGGAG	P	CATCAGCAGAGGTGGTCAATGGC	P	TTCTAGATGGCTGTGAGGTGGTGGG	GGGTGGCCGAGGCACTAGCTGTCTCTCTCAAGGGGAGAGAGCTCTGCAATG
704	1302	TGCTGTAGAAAGAGACGAGAGAG	P	GAATACGGTGGTCTCTCGAGTC	P	AATTCGAGTAACTCCGCTTTGGGAG	AATTCGAGTAACTCCGCTTTGGGAGGAGGAGAGAGAGCTGAGGCAATTTA
705	1303	AGAGATCATCTTCCACGCTTAAC	P	CAAGGAATTTCCATGCTGTTG	P	AAAAATAGTTAATCTGTTTCAGTTTC	AGCCCTCATTTGAGTCGATCTCTGACAGAACTGACAGATTAACATATTTTT
706	1304	AGATGACCCCTGATGGTTAGCT	P	AGGTGAATCGATCTGTGTGGTG	P	AAATTCAGCTAAACAAAATAACATCA	TTCAATTCAGGCTGTCTCAGCAGCTACCTGATGATTTATTTTGTATACGATTTTT
707	1305	CGTTTGTGTTTATGTTTAAAGACATCA	P	GTAATTCAGCACTTTTAGGACTCC	P	GATGCCAGCTAATTTCTCTTTTTT	GATGCCAGCTAATTTCTCTTTTTTAAAAAAAATTTTGGAGATCGGGCTCTG
708	1306	TGTAGCTGGGACTCAGGCGAT	P	AAITTTAAGCCTGCAAAACAGATTT	P	AGAAAGXATTCGATGCAAGCTATCTGT	CCCTTTCTTCAGAGGCGCAGACATGCCACAGATAGGCTGCGATCGAATCTGTT
709	1307	TGGCTTAGCCTTACTCTCTG	P	TCTGCTTGACTCTGAAATCACATTT	P	ATCACAATCTTGKCTACTATGCTATAA	GACTATAAAGGGTACATAAAATATTTACTTATACGATAGTACTAAGAATGTG
710	1308	AGGTGGCTCTATCTCAGAGAGA	P	ACGGTCTTGCTTCAATCTTAGACAGTT	P	CTATGCCCATTTCCAGTCACTAGTAG	AGTCCCAATTCACAGATCAGTAGAGAGAGAAATTTCTTAAAGCAACTGCTT
711	1309	GGGCTGACATATGTTATGTTAAGCAT	P	CNAGGTCACAAAGTCCAGGAC	P	AGCAGCAGGCGACAXACTGTCTATGTC	GAAITTCGCGAGGCTATGCTGGGATTCACACATAGACAGCTCTCGCTGCTTG
712	1310	TGCTTCCGACAGAGCAGC	P	CNAGGTCACAAAGTCCAGGAC	P	CTATGCCCATTTCCAGTCACTAGTAG	GAAITTCGCGAGGCTATGCTGGGATTCACACATAGACAGCTCTCGCTGCTTG
713	1311	GCACGCGAAGTCTGAAGTTT	P	TGTAGTTCTGAAGTGTGGGACTTTAAA	P	CAACAAAATGGTTCNAAAGATGAAATAT	ACAAAATTTGGTTCNAAAGATGAAATATGATATGATATATCTAAGAACCCCTTTA
714	1312	AAATCTCCAGACACAGAGAGAGC	P	AGAAATGAAGTGAAGAAACAAATTCGAG	P	TATAGACTCTGATGCGAGCAGCTACG	TATAGACTCTGATGCGAGCAGCTAGCGGTTTCACTTTGAAGAGCCCTCAATTTTC
715	1313	GTACAGACATATGGACGAGTGAAC	P	AGGAATGAGTGTACCATTTCTTTATCAC	P	TGTAAATTAATCTCTAANAAGAGATG	TGTAAATTAATCTCTAANAAGAGTGGTGTGGGGAATATAAGTGTGAATAG
716	1314	GCACATCTCCGCTCATTTG	P	GGCACCTGTAAATCCCACTTAC	P	TACTCGGCGACGACGCGCAGAGAA	AACTCTTGCCTCCAGAGTTCAAGTGTGTTCTGCTCTCTGCTCTGCCAGTA
717	1315	AAATTAAGTCAATATCTGATTTAAGATAC	P	CATGTTAGCCAGGATGCTGTC	P	GATCTCTGTGACTGCTGTGTCACCCAC	TAATCCCAACCCCTTTGGGAGCCCGGGGTGGTGGATCAGAGGTCAGAGGA
718	1316	TGACGACGAGGATGGGT	P	AGGCACAGCTGGAAGTCTTC	P	TTTCAATCTGACAGCAGGCTGCGAGT	CCACACTAGCAGACAGGCTGTGAGGCTTGAAGGGGAGGGGCTCTCCAGATGG
719	1317	TGAGGCTCTGGGTCTGC	P	CATGCGTAACAGGCGCCA	P	CTTGTGCTCACCAGCCGACXCTCAGAG	TTTCTGCTTTGGGCCCAACCCCTCAGGGTCTGGGCTCTGCGAGCTCTGA
720	1318	TTGAGGCGCAGAGTTTGTGA	P	CNAGTAGCTAGCAGTACAGATGTTGTC	P	TAGCCGACCTTATCTCTACAAAATAA	GCGAGACCCCTTATCTCTACAAAATACTAAAAAATTAGCCGGGATGGTGGTTC
721	1319	TTGAGAGGGGCAAGCATAT	P	CATGATTTCAAGGGGNAAGG	P	AGGAACTGTCAGCAGCTCTXTAATAAC	AGTTTTGTGGCCAGAGGACAGCTGTGGTTTATTAAGAAGCTCTGTCGAGGTTTC
722	1320	TTTTGTGACGAGTTTAAACAAAAGTA	P	ATTACAGGCTTGAGCAACA	P	CACCTGGGCTTTCATGTTCACTTAT	ACAAAAGCAATTTCTTAAAGGCTAACCAATGAACAAATGGAGGCCAGGTG
723	1321	CTGGCGTCTCCTCTGCTC	P	CCCTCCACTGCTGTACATAGTAGG	P	CTTCAACCATTTCCACTTCTTGGTG	CTTCAACCAATTTCCACTTCTTGGTG
724	1322	TGTTATTCGATATAAAGACTAGTTATGCA	P	CNAGAGTTTATGATGCCCATTAAGCTGA	P	GAAATGAATTAATTTGGCGCATGTGAC	GTGGGGGAGAGTGCAGACATGAAAGACTGACATTCGCAAAATTCATTCATTTC
725	1323	ATGACAGACTATGACATCATTAAG	P	GTAGTCCCACTATTTCAGAGATGCA	P	GTACAAATTTCAAGGTTCTCTCTATGG	CTCTGAGCAGTCAGAGCTTGTGTATACACATAGAGAGACCTGTGAAAATTTGATG
726	1324	TTCAACACTCTCCAGACAGC	P	CATATCCAGCCCTCTGTC	P	ACAGTATCTGTGTCMAATGTGMAACA	ACAGTATCTGTGTCMAAACTGCAACCGGCCAGAGGAGACACACCTGCCCTC
727	1325	ATCTCTCTGCTCTCAGCCTC	P	GNATGTCAGATTCGAGGTTTG	P	AGTGAGACTCTGTCTCTTAATAAAAA	CACCTACCTGGCTAAAGTTTAAAAAAAATTTTTTTTAAAGCAACAGACTCTCACT
728	1326	AGATTTGTTTCTAAGCTATCTCTAGATCTGT	P	CTGTGAGGATTAATAATAGTGAATGCA	P	ATTTTTTTCTGGGTCGCAATACGMAAT	ATTTTTTTCTGGGTCGCAATACGMAATTAAGAGCTTTTCTGTGTCGATTTCA
729	1327	TATAAAGGCTTATTTCAAGTTAGAGAGTGTTC	P	CAGTCTCGGGGCTGATC	P	GGTACAGNAATCCGATGACGACAGCT	CGTACAGNAATCCGATGACGACAGCCCTGTAGATCAATTCCTCCCTACG
730	1328	ACTCTGCTCTCTCTGCAAC	P	CTGGCTCTCCCATCTTTCAAG	P	AGAGAGTCCCTTAATCTATATCTGTT	CCTCGAGGCATCGAGCTTGGTGGMAACAGAAATTTATTTTAAAGATCTTTCT
731	1331	ATCTGACCTTATCTGTGTCMAACCC	P	CTTTTATTAAGTTATGGCTGTAGACAAAGG	P	TGTAACTCTTAATAAATAACTCTGTC	GTGACCCCTCGAGGCATGCAAGCTTTTAGAGACCGGAGTTTTTATTTAGGATTA
732	1332	ATCATCCAGGGGTGGG	P	CCTTACCTCTCTCAGATAGATATGCTTAC	P	AGNATAATGTTACGCTGACGCTGGGA	TAGAGTCGACTGCGAGCTTTCGAGGTTGGCAGCGCGAGGGTACGATATT
733	1333	ATGACAGTTTGAACATGATTAAGATTC	P	CTCCAGCAATCGCTGTTCTGAG	P	GGCAGGATTTGAACCCCTAACTCTGA	ATAGTTCGACCTCGAGGCATGCAAGCTCTCAGAGTTAGGGTTTCTAAATCTGGCC
734	1334	CCTAAGCCACTTTGGCAT	P	ATAGAGGCTCTCTGCTTTACC	P	TATACATTTCTATTAGTCACTTTT	TATACATTTCTATTCTAGTCAATTTCTCTCTGTTTCAGCTGGAGTTTCTGCTCT
735	1335	TCATGGGAGCTGTGTGTGG	P	CTTCCGCTCATGAACCTTTGGC	P	GGCCXCGAGTCAGGCTTCAATGAAGGAGG	GGCCXCGAGTCAGTCAATAAGGAGAGAGCTGACAGAGCTGGTTTCCAGCCCC

[illegible]

Row#	REF#	Upper PCR primer	1	Modified	Lower PCR primer	2	Modified gba primer	3	Flanking sequence	4
785	1393	CTAGAGGCGCCAGAACCTC			GCATTGATTAATAGGAATACAGATTT		P	CAAGCTCTAAGTACATCTAAGTCA	AGACTGTAAGTACATCTAAGTCA	AGACTGTAAGTACATCTAAGTCA
786	1396	TCCTCCATGCCGACAC			CATGAGGCGACTTAGCTCTTGAG		P	TCATGCTCTCAGGACCTGAGTTTGG	TCATGCTCTCAGGACCTGAGTTTGG	TCATGCTCTCAGGACCTGAGTTTGG
787	1397	CAAGTCCCTCTGAACCTGGAT			AAATGTGACTAGTCACTTACAAATGTGACT		P	GTGATGAGTCACTTACAGTGGACCTC	GTGATGAGTCACTTACAGTGGACCTC	GTGATGAGTCACTTACAGTGGACCTC
788	1398	AAATTTGAATCCAGGCACTCG			CTTTTCTACACTAGTCTAGCCCTCA		P	ATTACTCTTAACTGCTGATCACTCTCTA	ATTACTCTTAACTGCTGATCACTCTCTA	ATTACTCTTAACTGCTGATCACTCTCTA
789	1399	TACTAGTCTCAGAGTATTGTGTTGAAAG			TGTTGGCCCATACCTGCTAA		P	TGAATTGATTTGGGATATGGAAT	TGAATTGATTTGGGATATGGAAT	TGAATTGATTTGGGATATGGAAT
790	1400	TTTCATGCTGTGCTCGAG			GAITTTCTTCTACACAGAGCTCACTG		P	ACCATCCCATTCGGCTCTTCTACA	ACCATCCCATTCGGCTCTTCTACA	ACCATCCCATTCGGCTCTTCTACA
791	1401	CTGAGATTTATGATTTTGAATGAAAG			TGGCTCTACAGCTCAAGAGCTAGG		P	GGAGAAATATACCTTTTAGAGTTA	GGAGAAATATACCTTTTAGAGTTA	GGAGAAATATACCTTTTAGAGTTA
792	1402	AATATATGCTGTGATGCACTTTAAATACC			ATGGAATGGCCCTCTGCTGG		P	GCCAAACAGTCTTTCAGTCTTACAGTTAAG	GCCAAACAGTCTTTCAGTCTTACAGTTAAG	GCCAAACAGTCTTTCAGTCTTACAGTTAAG
793	1403	GTTTTATAGATTTGGGTAGATAAGG			CTTCTCTGCGCCATCCT		P	TGTGACCCCACTCTGCGCCACAG	TGTGACCCCACTCTGCGCCACAG	TGTGACCCCACTCTGCGCCACAG
794	1404	TAGGCTCTCAGCTAGACTCCT			CAGTGTCAATGAGCAGCAGG		P	TGATGTTGTGAGAGTGGAGAAAT	TGATGTTGTGAGAGTGGAGAAAT	TGATGTTGTGAGAGTGGAGAAAT
795	1405	ACCGGAGGTGAAGGCG			CAGKCTGGGAGCTGGG		P	AGCCCGCTCCGCTCTTGAAT	AGCCCGCTCCGCTCTTGAAT	AGCCCGCTCCGCTCTTGAAT
796	1406	TTTGAATCTTCCGACAC			GGCTGCGCAATCTTCTGTG		P	ACCGGAGGCTCCGAGCTGTCA	ACCGGAGGCTCCGAGCTGTCA	ACCGGAGGCTCCGAGCTGTCA
797	1407	GCTGGCGCAGGATCT			TTTGACAGCAAGACACTTCAATC		P	AACTAAACCCATTAATTTTGTGCTC	AACTAAACCCATTAATTTTGTGCTC	AACTAAACCCATTAATTTTGTGCTC
798	1408	ATGAGCTGCTCAGTTTCAATGGTTA			GTTTGTGCTGTGACACACTCTTCTG		P	TGAGTCAATGGAACCTCTGGAGGGTCA	TGAGTCAATGGAACCTCTGGAGGGTCA	TGAGTCAATGGAACCTCTGGAGGGTCA
799	1409	AGAGATTTGCTGAGGTTTAACTGT			CATGATCTTCCATCTCAGCG		P	CATTTAAACXAGAGGCGTGGCTCAAG	CATTTAAACXAGAGGCGTGGCTCAAG	CATTTAAACXAGAGGCGTGGCTCAAG
800	1410	AGTGTGTTGCTCCTCGGTACTT			GAACATGCTGCTGTGTCACCTC		P	AGGAGTGGTGTATCACTCTTAAAGAGC	AGGAGTGGTGTATCACTCTTAAAGAGC	AGGAGTGGTGTATCACTCTTAAAGAGC
801	1411	TAACTCAATTAACCACTCACTTAGG			CATTTTGGGCAACATCC		P	TGCTGCAAGCCCTCCAGTCTCACTG	TGCTGCAAGCCCTCCAGTCTCACTG	TGCTGCAAGCCCTCCAGTCTCACTG
802	1412	CTTTGCTGCTCCAGCTGAG			ACAGCAAAAGCAAAAGAACCC		P	CTGGTTTCCATGACCTGAGAGCT	CTGGTTTCCATGACCTGAGAGCT	CTGGTTTCCATGACCTGAGAGCT
803	1413	CAGTTATACCTTTAAATCTACTGGGC			GCCTTCCGCCCAATTT		P	GAATCACTGTTTCTGCAACATCAAAA	GAATCACTGTTTCTGCAACATCAAAA	GAATCACTGTTTCTGCAACATCAAAA
804	1414	CATATGCTAAGTATGATGATGAGTTA			AACTCTGCTCTTGGGCACTGTTAA		P	TGTTAATTAACATCTCTAGCCCTCA	TGTTAATTAACATCTCTAGCCCTCA	TGTTAATTAACATCTCTAGCCCTCA
805	1415	AACCAACCTCGCTGACA			TTTGAACCTCACTGCTTCCAGTT		P	ACATCAGAGGGCGGCAAGAGCTGAA	ACATCAGAGGGCGGCAAGAGCTGAA	ACATCAGAGGGCGGCAAGAGCTGAA
806	1416	AAGTAGTGGTCACTACTAATTAATGGAG			ACAGCAAGCAATCCCTCTCA		P	AACTCTTAACTATCAGATATCTCA	AACTCTTAACTATCAGATATCTCA	AACTCTTAACTATCAGATATCTCA
807	1417	CAATATGCTGAGTGTGAGATCA			TGACCTTAGTCTTCTTCCAGC		P	CGCTTAATTTCACTATTTGATAT	CGCTTAATTTCACTATTTGATAT	CGCTTAATTTCACTATTTGATAT
808	1418	CCTGACTTTCAGTAGGCAAG			TCCTCTGCTTATCTCTTCTCTGT		P	ATTGATTAAXAAATCTTATAGTAC	ATTGATTAAXAAATCTTATAGTAC	ATTGATTAAXAAATCTTATAGTAC
809	1419	AATGCAATTTGCTGCTTATTAAC			ACTTCCAGACTTGAATATAGGTGACTA		P	AGTCAACATTAACATTAATTTTAAAGT	AGTCAACATTAACATTAATTTTAAAGT	AGTCAACATTAACATTAATTTTAAAGT
810	1420	AGAACTACTACAGAGAGAAATTTGGTCA			CCCTCCTCCAGAGATGCTGCA		P	CCTCAGGAGATGATAGATGCTGCA	CCTCAGGAGATGATAGATGCTGCA	CCTCAGGAGATGATAGATGCTGCA
811	1421	CACCAAGATGAAATTTAGGTGAAAT			TGGGTTTATATTAGAAATGTTTAGGTT		P	CTGTGTCGAGGCGAGTAAAGAG	CTGTGTCGAGGCGAGTAAAGAG	CTGTGTCGAGGCGAGTAAAGAG
812	1422	TTAGAGGAGTCCAGTATTAACCTAT			TGACAGGCTAGAGAGCCAA		P	ACAAGTAGAGCTTTTCAATTTATTA	ACAAGTAGAGCTTTTCAATTTATTA	ACAAGTAGAGCTTTTCAATTTATTA
813	1423	TGAGCAAAAGTTTAAACAAACCA			CACCTGGGCTCAGGAC		P	AGGGGTTGTGAGGCGAGGGGCTG	AGGGGTTGTGAGGCGAGGGGCTG	AGGGGTTGTGAGGCGAGGGGCTG
814	1424	ATCTCTACAAAAGACAAATATAGCTG			GCTCAGCGAGATTTGAGC		P	ACCTCCGAAATTCAGGTGATCTCCCG	ACCTCCGAAATTCAGGTGATCTCCCG	ACCTCCGAAATTCAGGTGATCTCCCG
815	1425	TACTTATTTCTATCCGAGCTGTGA			CAGTCTATTTTCCGCTTTTC		P	CTTCTTTGCGGCTCCCACTTCA	CTTCTTTGCGGCTCCCACTTCA	CTTCTTTGCGGCTCCCACTTCA
816	1426	TCAACTGCAATTTGCTTCA			CAGAAATGCGAAATTAATAAAT		P	AGAAATGCGAAATTAATAAAT	AGAAATGCGAAATTAATAAAT	AGAAATGCGAAATTAATAAAT
817	1427	CTAAACATAGTTTGGTCTTGTAGTT			TGTACAGATGTTTATCAGAGATATGTC		P	ACAGCATATGTCAGAGTGGTTTGA	ACAGCATATGTCAGAGTGGTTTGA	ACAGCATATGTCAGAGTGGTTTGA
818	1428	TAGCAAGCATCAGTACAGCACTAA			TCAAAATTAATGCAAAAGTGAACCA		P	TTACTCTCAGATATTTGTTATTTGCT	TTACTCTCAGATATTTGTTATTTGCT	TTACTCTCAGATATTTGTTATTTGCT
819	1429	CGAGTAGGTTATGATAGCTTTTAACTATTC			TCCTTGCCCATTTCTTTTACC		P	ATATGCTTCTTTTAGGAGCTTA	ATATGCTTCTTTTAGGAGCTTA	ATATGCTTCTTTTAGGAGCTTA
820	1430	TTTCTCTCTTAAATGATTTGAGC			ATAACTCTTCAGAAATTAAGAGAAATGTA		P	GTGACXGTCTAGTTAATACAGACCA	GTGACXGTCTAGTTAATACAGACCA	GTGACXGTCTAGTTAATACAGACCA
821	1431	ACGAGATCTCAAAATCTTCAAAAG			CTTTGCGCATTTCTTTTACC		P	TTGGCTCXACAGTCTGGGATTA	TTGGCTCXACAGTCTGGGATTA	TTGGCTCXACAGTCTGGGATTA
822	1433	AAAGGAGAGAGAGTGG			CTTACAGTCACTGCTCGG		P	GTGCTAGTAAACCAAGTAAACCTGA	GTGCTAGTAAACCAAGTAAACCTGA	GTGCTAGTAAACCAAGTAAACCTGA
823	1434	ATCTTCTGAGCGCGAGTTT			CCTTAGTTTCTTACGTCTTTTATTTA		P	CAACTXCATTTCAAGCTGGGCAACA	CAACTXCATTTCAAGCTGGGCAACA	CAACTXCATTTCAAGCTGGGCAACA
824	1435	TOMACACTCTATCCGAGAC			CAAAAATGTTTTCGCAATTAAC		P	GAGGCTCAGCGTGTGATATGAG	GAGGCTCAGCGTGTGATATGAG	GAGGCTCAGCGTGTGATATGAG
825	1436	TACTAATAACAAAGTTAGCGGG			CTCGCTCCGAGTTCA		P	AGCATTTCTCTGCTCXGCTCT	AGCATTTCTCTGCTCXGCTCT	AGCATTTCTCTGCTCXGCTCT
826	1437	CCCTTATGCGCAATGTTCTAGACTA			AGACTCAAAAGAAATATATCAGG		P	TTCCAAAAGAAATATAGAGAGTTAT	TTCCAAAAGAAATATAGAGAGTTAT	TTCCAAAAGAAATATAGAGAGTTAT
827	1438	TGTACAGACAGGATGCGCA			GGATACCTTTTCTTCTTGTAGTGG		P	ATACTATTTGAAATAGAGTAGTGA	ATACTATTTGAAATAGAGTAGTGA	ATACTATTTGAAATAGAGTAGTGA
828	1439	AAATACTGAGCTAGAGAGCTGTC			CAAAAATGTTTCTTCTTCTTATTTA		P	AGACCCAGGCTCAGGTCAAGTTCTT	AGACCCAGGCTCAGGTCAAGTTCTT	AGACCCAGGCTCAGGTCAAGTTCTT
829	1440	CATGAGTGTGATGACGGC			CAAAAATGTTTCTTCTTCTTATTTA		P	GAATAGAGGAGCTGCAATTAACAT	GAATAGAGGAGCTGCAATTAACAT	GAATAGAGGAGCTGCAATTAACAT
830	1441	AGAAAGCTGTGATTTTAAAGTTTCA			CTCGAGGCTGTGCAAGTTATG		P	GGTGAAGCTTAGATACAGAGCTG	GGTGAAGCTTAGATACAGAGCTG	GGTGAAGCTTAGATACAGAGCTG
831	1442	TAACTAACAATAATCTGCGAG			GGCCCTCTTAACACACTCT		P	CCCAAGCTGAAATACACAGAGTTT	CCCAAGCTGAAATACACAGAGTTT	CCCAAGCTGAAATACACAGAGTTT
832	1443	ATTATTAATATCCCAACGGAG			CATTAACAGACACACAGGGTGG		P	CCAGGTTGGCTATCTCTGAGTCCA	CCAGGTTGGCTATCTCTGAGTCCA	CCAGGTTGGCTATCTCTGAGTCCA
833	1445	CTTTGGCATAGTAGGAGTCAATATT			TTAATGTCACACAAATAGAGTCAACACT		P	GAAGTCAACAGTACCAAAAACCCATA	GAAGTCAACAGTACCAAAAACCCATA	GAAGTCAACAGTACCAAAAACCCATA

Row#	REF#	Upper PCR primer	1	2	3	Modified	Lower PCR primer	Modified	CSA primer	Flanking sequence
834	1446	GACGAGCTCATGATTTTGGAAACC	P				TGAGGCGAGCGCTTGTA		GCTAGTCACTGTTTTTTTTTTGTTG	ACAGGCAAAAAAAAAAAAAAAAAA
835	1447	AAATAAATAAGCGAGGATCATAGAA	P				TCAAGAGATACGATTTAGCCTTTAT		CTATTGCTTGTAATCTCATTTTCAT	ACATTTAGCAAAAAACAGACTGAA
836	1448	TTCAAGCCCTCTTCAGTTC	P				CAAGAATCOMAGATCAGTGTGA	P	GGATGAAATGAGTGTCCAGAGCGG	GGATGAAATGAGTGTCCAGAGCGG
837	1449	ATATTATTGAAGATAAGCGATATATGCTATTAGG	P				TGTGCCATTTTTTGCATTCCT		CGATTCTGACCATCTTCAATAGGTGG	AAATTTCAAAAAATGAATACTAT
838	1450	AAATTTTATAGAGATATATAGGAGCTC	P				AAAAATTCACAGTCTCTCAACATTTC		ACCATTTCCITTTAGCAATGCGCCAT	TAGTTAAATTCATCATAGTCTATG
839	1451	TCGACAGCTGGGCAAGA	P				GTAGTGAGAGTAGTACCTCTGAACCCC	P	AGTGTGATAGAGTAGTACGAGCATGA	TGTGATAGAGTAGTACGAGAGT
840	1452	GTGCGATCTGGGCTCACT	P				CATGCGCTGATCCGAC		TACTGGGAGGCTCGAGCGACAA	AGTCTCTGCGCTCTGAGTCTCA
841	1454	ATTGATATTCGACAGGAAACAA	P				CTCACTCTGCTGCGCTTCC		CCCTGGCTAAATATCTCAAAGAG	TTCGCTTTCCCTTTGAAATTTGA
842	1455	TTCTAGAGAGCGGGCTAG	P				GACTCAAGAGAGAGACTCAGGATC		AGTGAGCAGACGACGAGAAACAG	TTCTGGTTGACCCCTTCCGACCCC
843	1456	CCACACAGCAATCTACATACGAT	P				TGCTTAATATTTATTAAGCTCTATAACATCA		TATAACCATGACTTGTGAGCAGTAA	TTTTGCTCTCTGTGATGTATG
844	1457	CTAGCTCGTACCTGCACAC	P				TGCTTTCTCTGTAGAGCTCTG	P	TTGAATAAGATTTCTCTCAAGAAAT	CAATAGATTTCTCTCAAGAAATA
845	1458	TTATTGAAGCCCTCTGCAC	P				CAGGATCTCCGAGGTGCATC		CATCCAAATATGTCTTTGTCACCTT	CCCAAGTATCCCAAGCGCCCTCT
846	1460	TAAATCCGAGCTCTGGG	P				GTATTATTAGAGCCAGTCTCACTCA	P	GCGGAGCAATGCGCTGCAACGAGGA	GCGGAGCAATGCGCTTGAACAG
847	1461	GTGGCAAGGCTCTTCACACTAG	P				AGAAGTAAATGCTGCAAAAAATGAAATATG	P	CCATCTGTAGACTGTCCAAATATGTAT	CATCTGTAGACTGTCCAAATATG
848	1462	TTGAGTCAGTTGCTGAAATTC	P				GGCACCCCTATCTTTGACAC	P	TCTAGTGAATATTAATAATCTAAGCA	TCTAGTGAATATTAATAATCTA
849	1463	ACCAATTCGCAAGAGCTTAAG	P				GTAGACGCCCCAGTTTTT		ATTGTAGTTCAATTTGACCTCTCCA	TGACACACCGCGATGCCCCGGA
850	1464	ATTAACCTCAGGATATGTTGCT	P				CTATTGACGAGGAGATCGGAGG		CCAGCGAGGCGCACAGCAGAGAGCG	ACAGCTGGGTGAGTTTGGCAGCG
851	1466	TTGCGAGACTCATGTGATCTAGAG	P				GAATATCTTGGGACACAGCACTG		TGCGMAGATTTGACATAGTAA	TGCGMAGATTTGACATAGTAA
852	1467	AGCGAGCTAGTCCACCTC	P				CATCCGCTAAATTTAGCGATGCTA		TCCAAAGCAACAATCATAAAGAAACAC	TGCGTTTCTCTCTTTTCCGACAG
853	1468	TCATTTCCAGGCTGATGCG	P				AAAAAGAAAGAAAGAAATTTGGC	P	TGTAAGCAGGCGAGCACTCTGGGT	TGTAAGCAGGCGAGCACTCTGGG
854	1469	AGGAAATTCAAATCTGGAACAGAC	P				TGTCATTTACTGCCAGAAATTAAGCTG		AAATCGCCTCGAGATTGCTTTTCTTC	GGCTCAGGAAAGAAATTTCA
855	1470	CTTTGATCATATACGTTGCATGAGAA	P				CAGACATTTAAAAATCTCTATTTTACATAGG	P	AGCAACAATGCAAAATAGAAACCGAA	AGCAACAATGCAAAATAGAAAT
856	1471	AGGTGCCCACTCATCTGCG	P				GGGAGCGCAGCGCTCTTCT	P	CCCAATGACCAACAGTGTCTGTCTC	CCCAATGACCAACAGTGTCTGT
857	1472	TACTTGCATGAGCAATGTGAG	P				CTACTTTAGCGAGGACCGAA	P	GTCCATCCATCTCTATCAGCAATCT	GTCCATCCATCTCTATCAGCAAT
858	1473	TTATGMAAAACCCACAGCOMATAT	P				CATGATCATACCTGTTCCTGG		AAATTCATTCCTCCATTTTCTTAGTGT	TGMAAACTGGCACAGACGGA
859	1474	TTGCTATGCTTAGGTTGTGA	P				GTAGCCTCTGTCTGTGGGCC	P	AAACCTCATGTCGTCGCAAAATCAAA	ACCTCAGTGTGTCGCAAGATCA
860	1475	CTTTGATGTTTATTCATATGACAGC	P				AGTACGAGATTTTCAGTTACATAGTGAAC	P	TGTTTAAACACCGCAGTATGTCCA	TGTTTAAACACCGCAGTATGTCCA
861	1476	ACGTTCTTAAGCACTTTTCTGCA	P				AAAGTCAACGATTCAGAGAGCCA		TATCAAAAAATCTCATAGGCTCAGCG	TCCCTCAACACATCTTCTGTGCG
862	1477	TGAGGCGCAGGAGGAGC	P				GAAACTGAGTCTGGATAGGCGTAG	P	AGGACAGXGATCTCTGATTTCTCTAA	GGACAGATCTCTGTGATTTCTCT
863	1478	TGTGCTGAGCGAAGATAGCTACA	P				CAATTCGCAATTAAGAGCGCTAAT		TTGATTTTCAGATTTTCTGTTTTTT	AAGAGTCAAAATCTGTCTCAGAA
864	1479	TTTAGCTTTTGGCGATGAC	P				GGCAGGAGCTTGTGGAT	P	GTGCCGGGATGCGCTGATCTATGCG	GTTTCTGTCTACTCCAGCTCCA
865	1480	CTCATAAATTTCCATATTTTCTCTGTACT	P				AGNAAACCTGGCTCAGCAAA	P	GTTAGAGTGACAGATTTTTTTTTTT	GTTAGAGTGACAGATTTTTTTTT
866	1481	TGATGAGCAGGCGATAAG	P				CTCACTACCCCTGGCCC		CTCAGATTTATTTCCACCGCCCTC	GTTTATGCTTTTGTGATCAGAT
867	1482	ATTCCTGTATACTGAGAGCTGACC	P				CTTTATTTTCCACCTGTTAGACATTC		ACAGXGATTTTGGCGAGTTGTATTTAC	GGAAAAACATTTACTAGTTTCA
868	1483	TTTTTGAAATGTTTTTGGCCA	P				GGAGCGAGCAAAAAGTCCC	P	TTAAATCTGAGTGTAAACATTCGTAGAA	GGCTCGAGCTTCCAGCATAGTCA
869	1486	AAATCTAGCTGGTACGAGCCGG	P				CTTAACTTGTGAACACAGCCGAG	P	CTTTTGAACAGCACTGCTGTGCAATC	AAATCTGAGTGGAAACATCCG
870	1487	GCAGCTGTCTGACTACCGG	P				CAATTAACAGCACTGCTGTAGCAAA	P	ACTGTTCTAGAGTTGAAACTTAAGCC	TTGATGCACACACTGCTGCTCA
871	1488	CAATTCACAGATCGAGTAGAGTGA	P				AAATACTTGTAATTTCTTGGACTTTTCTCTTT	P	CAGCTGTGATCTCTCAGAAATTA	ACTGTTCTAGAGTTGAAACTTA
872	1489	CACACAGTAAATATTCAGAGCTTTTAA	P				CAGGCTGAGACAAACCCATCA		ACGGAAGAATTTAGACAGGCTAC	ACTGTTCTAGAGTTGAAACTTA
873	1490	TCTCTGCCCTTAATCTAGACGATATCT	P				CAGGTGGAACAGTGGAGACA		TCATTTCACTACCCCTTTTCTGCTGTC	ACTGTTCTAGAGTTGAAACTTA
874	1491	TCACACAGCAGAGCAAGAGTG	P				CCCTCTCAGCATTTAGCGTTCTGCG	P	GMAATCTTCTATCTAGTATCGCTGAGT	TCATTTCACTACCCCTTTTCTG
875	1492	TCCTTAGAAGTATCTTTGGGATATAGG	P				CAGAGAAGGCTCGGCTAG	P	CCAGCGCTCTGGGAG	TTGACATGTGGAGAACTCTCT
876	1493	TTGTCTAAGTGTCTCTGTAGAGAG	P				ATGCTGGGTGTAGGTGGCT	P	GTTTCTTCCGACCCCTCCXGCTCTAG	GGCTCGCTTGGAGAACTGGA
877	1494	AGGCTGTTAGCCTGGG	P				CATCTGGGCTGATGGTGC	P	GACAGATAAACACATGTGGCCATCTT	CCAGATAAACACATGTGGCCAT
878	1496	CTCCCAGGCTGTGAATTT	P				AGATTTGGTGGCGCAAGAGACA		CTGTAACCTGTACATCTTTATTTTC	GACMAAACTGTACATCTTTTCT
879	1497	ATACACACACATCTAACCCAGGA	P				TCCTGTCTAGGTTCTGGGATC		CCTAAATCTCTCTGAGCCGCTTTC	ATGACATCCACATCTGGCCAT
880	1498	GAGAGTTTATCTGCTCTTTTGGTCTA	P				CCAGGTGTCAAAAAACCTATACTATATCTC	P	TAAGATCATATAGGGGGCTGTGAGCC	TAGATCATATAGGGGGCTGTGAG
881	1499	AGAGATTATTTCAGACACGAATAGAG								
882	1500	TGGAGGAAGAGGTAATTAAGGTTTTG								

[illegible]

Row#	REF#	Upper PCR primer	1	Modified	Lower PCR primer	2	Modified GBA primer	3	Flanking sequence	4
932	1553	CCGAGGAGAGACAGTCAGGA		P	TTAAACATTAAACGACTCTCTTCTC		AGCKCACAGAGGAGAGCGGACACTCA		ACTGGAGGGCCAGAGAGGGCTAGCTGAGTCTCGGCTTCTGCTCTGTCG	
933	1554	TGCTTCTGCCCCACAGC			CATCACAGTTTCTGAGGCC		TATCAGAGGAGCTGGCTTTTTCACACCA		TCAGAGGAGGTGGCTTTTTCACACAGGGCAATATTTTAAATCAAGAGTGTG	
934	1555	TTGGCTGCTGCGGTGGC		P	CACACTCCCATGAGGAGT		TGACAGAGCAGTGAAGCCAAAACCCC		TCAAAACATCAGCTTTTTCAGGGGTGGGGCTTTTGCGCTGACTGCTCTGTCA	
935	1556	AAGCCTCAACTATATACTGCTCTG			GATTTCATTTCCATCTCTTTTCTCTAG		GTGGATATATTGAATCMAATCMAATCAGGC		CGATATATTGAATCMAATCAGGGTGGCCCTTGGCCCTCATAGAGCTAG	

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which
5 is described and claimed.

We claim:

1. An oligonucleotide comprising a nucleic acid sequence selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+3, or complementary sequences thereof, wherein n=0 through 934.

2. The oligonucleotide of claim 1, wherein X, when present in said oligonucleotide, corresponds to a single-nucleotide-spacer selected from the group consisting of anucleosidic moieties, abasic moieties, non-naturally occurring nucleotide analogs, and non-Watson/Crick base moieties.

3. An oligonucleotide comprising a nucleic acid that hybridizes with a first portion of nucleic acid selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+4, or complementary sequences thereof, wherein said first portion to which said oligonucleotide hybridizes also hybridizes with a nucleic acid selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+3, and wherein said oligonucleotide does not hybridize with a nucleotide base on said SEQ ID NO:4n+4, or complementary sequences thereof, corresponding to the nucleotide immediately adjacent the 3' end of said SEQ ID NO:4n+3, wherein n=0 through 934 and n is the same value for both SEQ ID NO:4n+4 and SEQ ID NO:4n+3.

4. An oligonucleotide comprising a nucleic acid that hybridizes with a first portion of nucleic acid selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+4, or complementary sequences thereof, wherein said oligonucleotide does not hybridize with a second portion of said SEQ ID NO:4n+4, or complementary sequences thereof, wherein said second portion of said

SEQ ID NO:4n+4, or complementary sequences thereof,
hybridizes with a nucleic acid selected from the group of
SEQ ID NOs consisting of SEQ ID NO:4n+3, and wherein said
second portion of said SEQ ID NO:4n+4, or complementary
5 sequences thereof, also includes a nucleotide base on
said SEQ ID NO:4n+4, or its complement thereof,
corresponding to the nucleotide immediately adjacent the
3' end of said SEQ ID NO:4n+3, wherein n=0 through 934
and n is the same value for both SEQ ID NO:4n+4 and SEQ
10 ID NO:4n+3.

5. An oligonucleotide comprising a first
portion of nucleic acid selected from the group of SEQ ID
NOs consisting of SEQ ID NO:4n+4, or complementary
sequences thereof, wherein said oligonucleotide excludes
15 a second portion of said SEQ ID NO:4n+4, or complementary
sequences thereof, wherein said second portion of said
SEQ ID NO:4n+4, or complementary sequences thereof,
hybridizes with a nucleic acid selected from the group of
SEQ ID NOs consisting of SEQ ID NO:4n+3, and wherein said
20 second portion of said SEQ ID NO:4n+4, or complementary
sequences thereof, also includes a nucleotide base on
said SEQ ID NO:4n+4, or its complement thereof,
corresponding to the nucleotide immediately adjacent the
3' end of said SEQ ID NO:4n+3, wherein n=0 through 934
25 and n is the same value for both SEQ ID NO:4n+4 and SEQ
ID NO:4n+3.

6. An oligonucleotide comprising a nucleic
acid sequence selected from the group of SEQ ID NOs
consisting of SEQ ID NO:4n+1, or complementary sequences
30 thereof, wherein n=0 through 934.

7. An oligonucleotide comprising a nucleic
acid sequence selected from the group of SEQ ID NOs

consisting of SEQ ID NO:4n+2, or complementary sequences thereof, wherein n=0 through 934.

8. A pair of oligonucleotides comprising two nucleic acids selected from the group of SEQ ID NOs
5 consisting of SEQ ID NO:4n+1 and SEQ ID NO:4n+2, or complementary sequences thereof, wherein n=0 through 934, and wherein said primers correspond to two consecutive SEQ ID NOs having the same value for n.

9. A triplet of oligonucleotides comprising a
10 nucleic acid sequence selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+1, SEQ ID NO:4n+2 and SEQ ID NO:4n+3, or complementary sequences thereof, wherein n=0 through 934, and wherein said oligonucleotides correspond to three consecutive SEQ ID NOs having the
15 same value for n.

10. A kit comprising at least one oligonucleotide of claims 1, 3, 4 or 5.

11. The kit of claim 10, further comprising two amplification primers.

12. The kit of claim 11, wherein said primers
20 comprise a nucleic acid sequence selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+1 and SEQ ID NO:SEQ ID NO:4n+2, or complementary sequences thereof, wherein n=0 through 934, and wherein said three
25 oligonucleotides correspond to three consecutive SEQ ID Nos having the same value for n.

13. A kit comprising an oligonucleotide, wherein said oligonucleotide comprises a nucleic acid sequence selected from the group of SEQ ID NOs consisting

of SEQ ID NO:4n+3, or complementary sequences thereof, wherein $n=0$ through 934.

14. The kit of claim 13, further comprising two amplification primers.

5 15. The kit of claim 14, wherein said primers comprise a nucleic acid sequence selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+1 and SEQ ID NO:4n+2, or complementary sequences thereof, wherein $n=0$ through 934, and wherein said three oligonucleotides
10 correspond to three consecutive SEQ ID NOs having the same value for n .

16. A kit comprising the two oligonucleotides of claim 8.

15 17. A kit comprising the three oligonucleotides of claim 9.

18. A method of genotyping a nucleic acid sample comprising hybridizing the oligonucleotide of claims 1, 3, 4 or 5 to the nucleic acid sequence selected from the group of SEQ ID NOs consisting of SEQ ID
20 NO:4n+4, or complementary sequences thereof.

19. The method of claim 18, wherein said oligonucleotide is employed in a primer extension reaction.

25 20. The method of claim 19, wherein said primer extension reaction is a single-nucleotide primer extension.

21. A method of genotyping a nucleic acid sample comprising performing a primer extension reaction employing the oligonucleotide of claims 1, 3, 4 or 5.

22. The method of claim 18, wherein said
5 primer extension reaction is a single-nucleotide primer extension.

23. A method of genotyping a nucleic acid sample comprising performing a primer extension reaction employing an oligonucleotide comprising a nucleic acid,
10 or fragment thereof, selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+3, or complementary sequences thereof, wherein n=0 through 934.

24. The method of claim 23, wherein said
15 primer extension reaction is a single-nucleotide primer extension.

25. The method of claim 23, wherein said fragment excludes from 1 up to 10 nucleotides from the 3' end of said SEQ ID NO:4n+3.

26. A fragment of SEQ ID NO:4n+3, wherein n=0
20 through 934, wherein said fragment excludes from 1 up to 10 nucleotides from the 3' end of said SEQ ID NO:4n+3.

27. A method of genotyping a nucleic acid sample comprising:

25 a) amplifying a target nucleic acid sequence that hybridizes to an oligonucleotide selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+3, or complementary sequences thereof; and

- b) performing a single-nucleotide primer extension reaction employing an oligonucleotide comprising a nucleic acid selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+3, or complementary sequences thereof,

wherein $n=0$ through 934 and is the same value in both steps a and b.

28. A mixture of reagents comprising:
- (a) 10 to 200 mM buffer, having a pH ranging from 6.0 to 9.0;
 - (b) 15 to 250 mM monovalent-cation salt;
 - (c) 0.5 to 25 mM divalent-cation salt;
 - (d) 0 to 0.02% volume exclusion agent
 - (e) 0.25 to 2.5 mM of each of dATP, dCTP, dGTP and dTTP.

29. A method of amplifying a nucleic acid sample comprising the steps of:

- (a) combining two amplification oligonucleotides with said nucleic acid sample;
- (b) adding to said nucleic acid a reagent comprising:
 - (i) 10 to 200 mM buffer, having a pH ranging from 6.0 to 9.0;
 - (ii) 15 to 250 mM monovalent-cation salt;
 - (iii) 0.5 to 25 mM divalent-cation salt;
 - (iv) 0 to 0.02% volume exclusion agent
 - (v) 0.25 to 2.5 mM of each of dATP, dCTP, dGTP and dTTP.

(c) contacting said nucleic acid with a nucleic acid polymerase.

30. An oligonucleotide comprising a nucleic acid sequence selected from the group of SEQ ID NOs
5 consisting of SEQ ID NOs: 1-3740, or complementary sequences thereof.

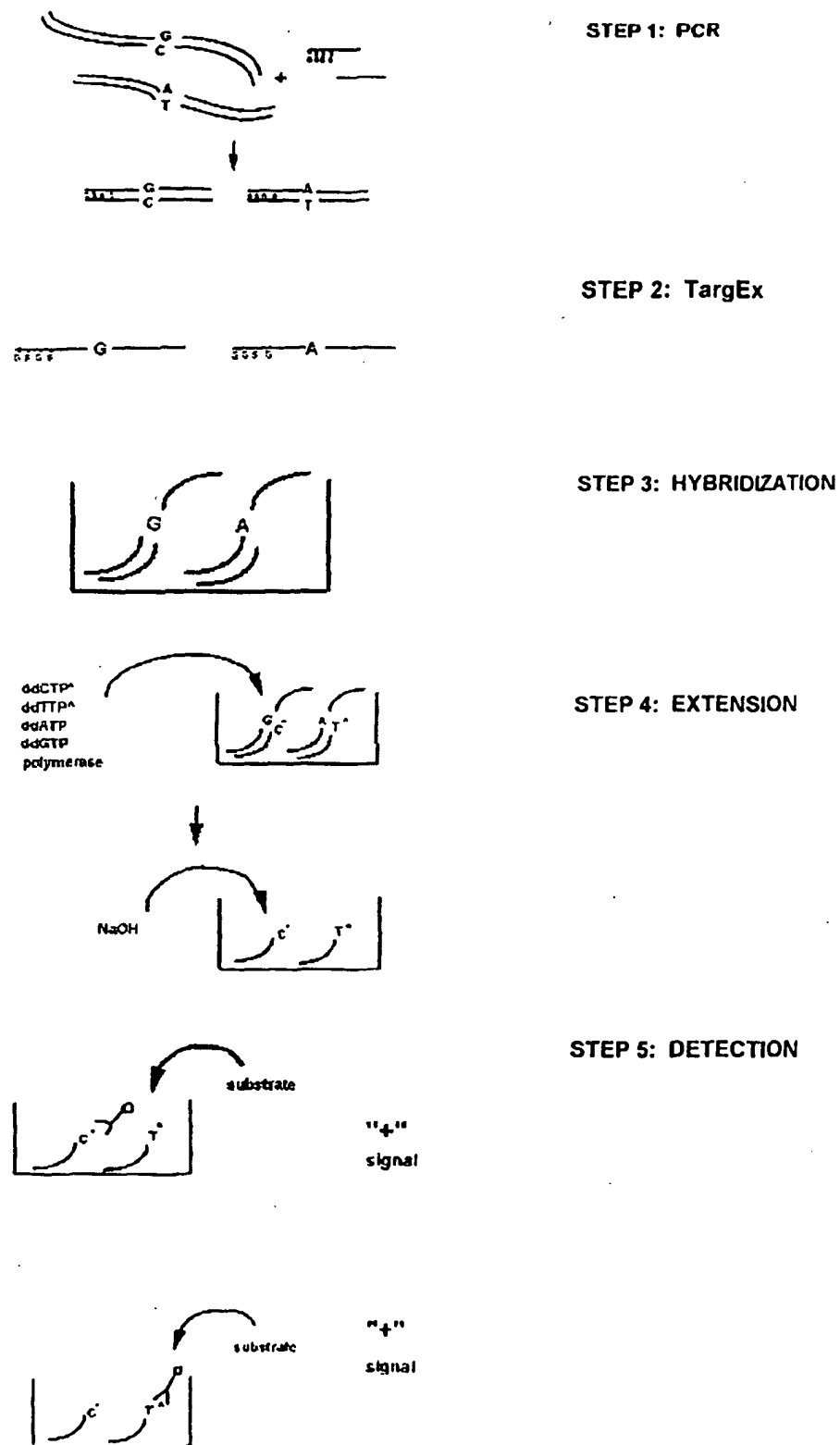
Diagram of GBA Process

Figure 1

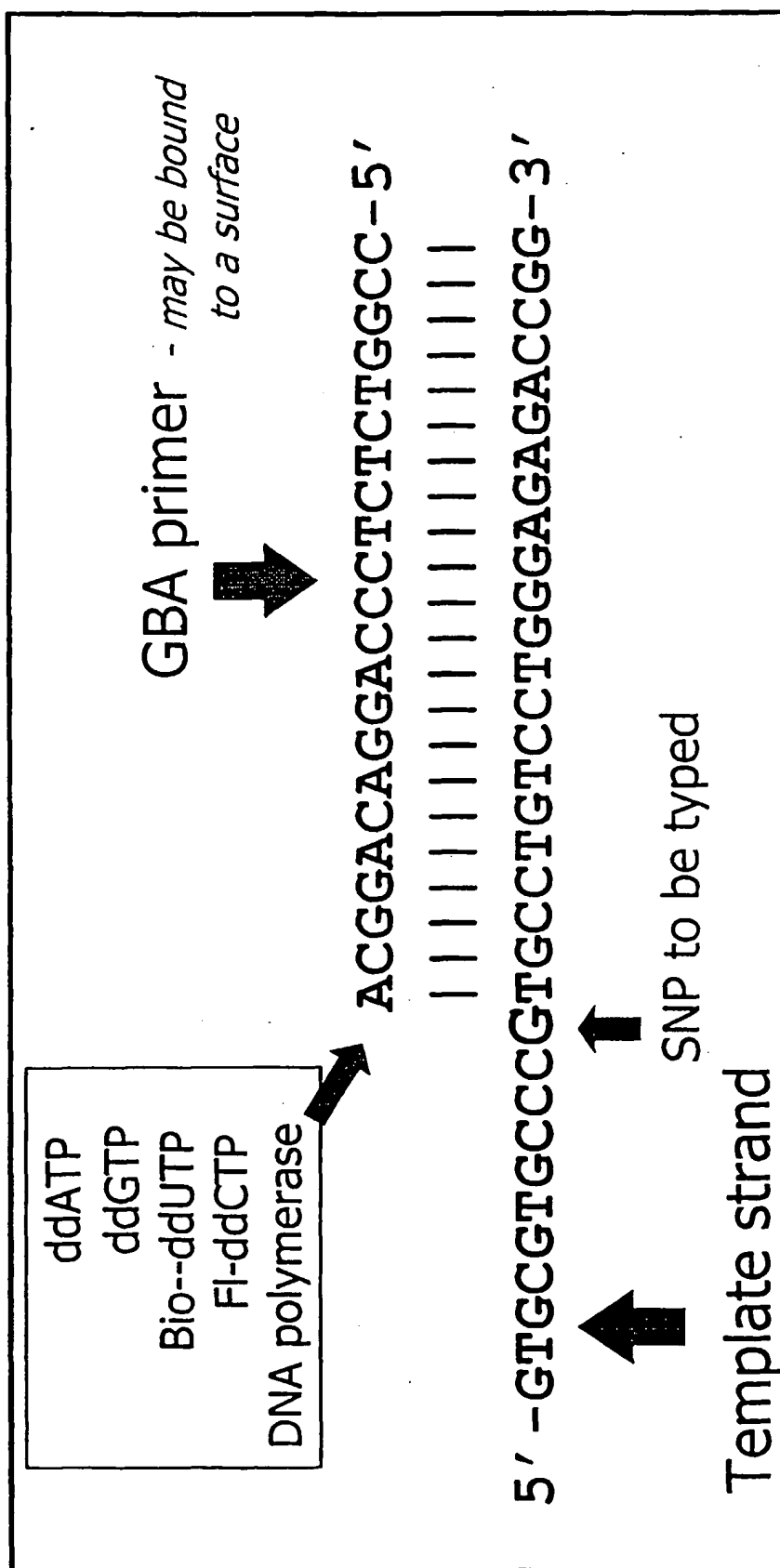


FIGURE 2A

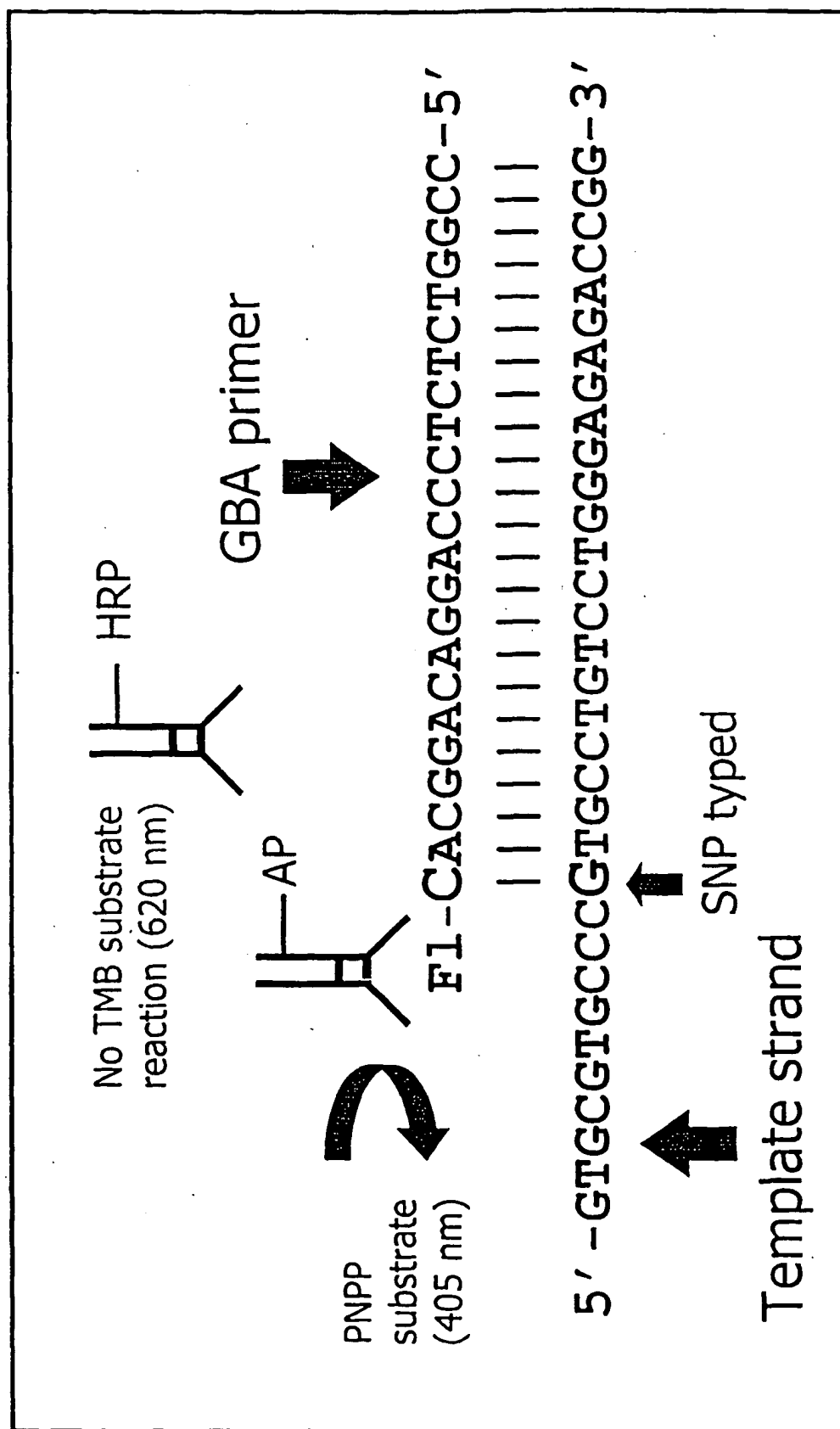


FIGURE 2B

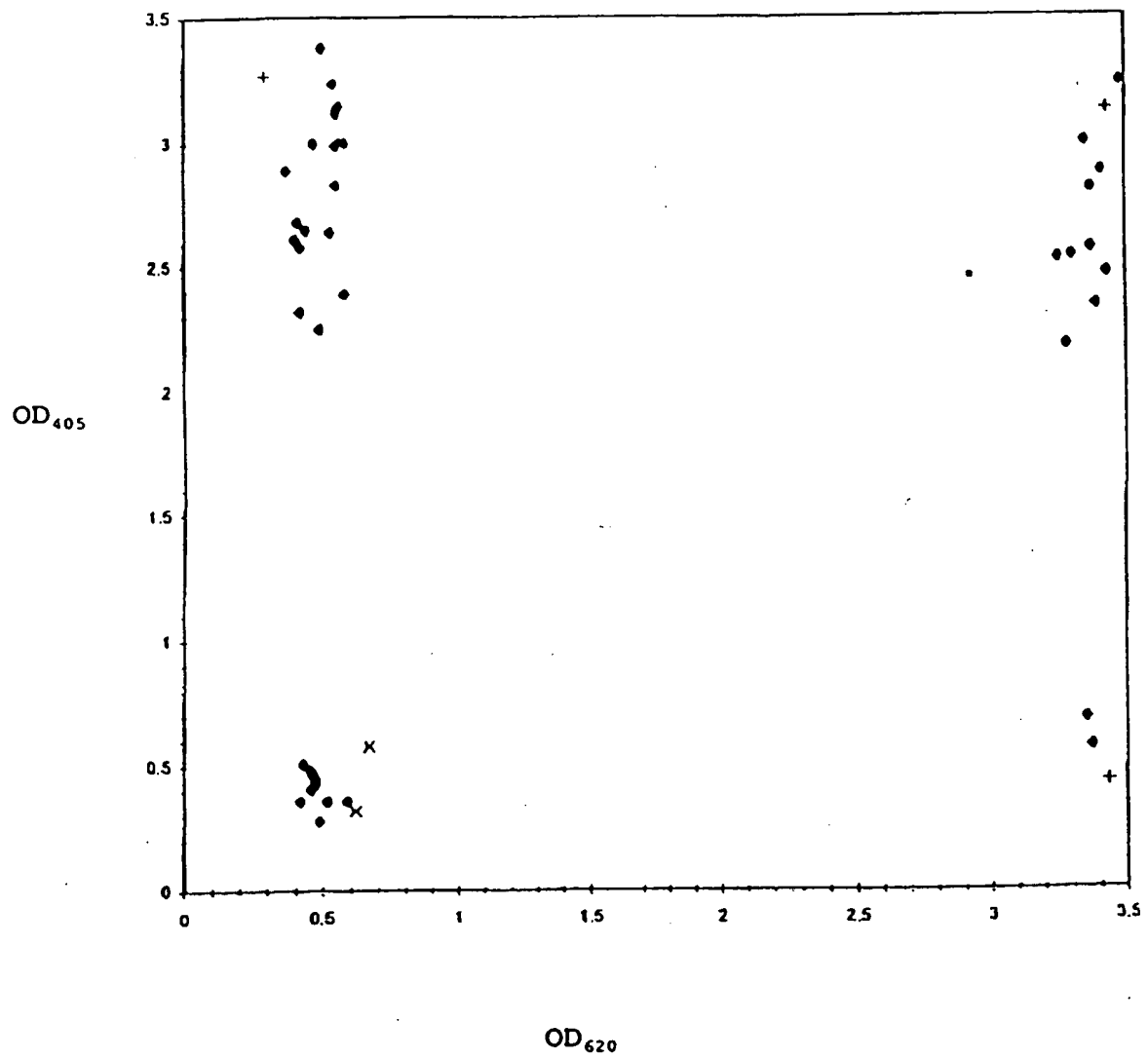


FIGURE 3